सत्यमेव जयते

INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

MGIPC—S1—6 AR/54—7-7-54—10,000.

TRANSACTIONS OF

THE ROYAL SOCIETY OF CANADA

SECTION V
BIOLOGICAL SCIENCES

THIRD SERIES—VOLUME XLI—SECTION V

MAY, 1947

OTTAWA
THE ROYAL SOCIETY OF CANADA
1947

TABLE OF CONTENTS

Fluctuations in Animal Populations with Special Reference to Those of Canada. Presidential Address, by J. R. Dymond, F.R.S.C. 1

Effect on Growth and Basal Metabolic Rate of Excessive Amounts of Some of the B Vitamins in the Presence of Added Desiccated Thyroid. By John Allardyce, Ewald Goranson, and Edward Singer 35

Utilisation digestive et teneur en matières cellulosiques de la ration. Par André Gasnier 49

Lethal Temperature Relations of Certain Fish of the Toronto Region. By J. S. Hart 57

Serious Mortalities in Prince Edward Island Oysters Caused by a Contagious Disease. By A. W. H. Needler, F.R.S.C., and R. R. Logie 73

The Deaminase Activity of Proteus Ichthyosmius. By Nora E. Neilson and Blythe A. Eagles 91

Variations of Thermogenesis during the First Stages of Seed Germination. By Henri Prat 95

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLI : SERIES III : MAY, 1947
SECTION FIVE

PRESIDENTIAL ADDRESS

Fluctuations in Animal Populations with Special Reference to Those of Canada

By J R DYMOND, F R S C

WHILE the populations of many animals appear to remain relatively uniform over considerable periods of time, wide fluctuations characterize the populations of many others and in no country are such fluctuations more marked than in Canada.

The varying hare *(Lepus americanus)* has become an almost classic example of a species with a violently fluctuating population (Fig. 2). MacLulich (1937) estimated that in some habitats hares decreased from over three thousand per square mile to near zero in less than one year. Return to abundance in the case of the hare takes place much more slowly than does the decrease but violent fluctuations in this species have occurred at remarkably regular intervals for as long as our records extend in the past.

Another classic Canadian example of a widely fluctuating population is that of the sockeye salmon in the Fraser River. Before the curtailment of its reproduction through the partial blocking of the river by a

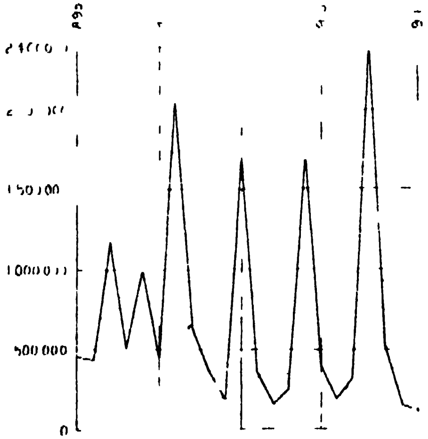


FIGURE 1.—Periodic fluctuation in abundance of Fraser River sockeye salmon, as represented by cases of canned salmon packed each year, 1895-1916 (British Columbia, Report of the Fisheries Department for 1944).

rock slide at Hell's Gate Canyon in 1913 the population as measured by the number of cases of canned fish had fluctuated between a high of nearly 2,400,000 in 1913 to a low of scarcely more than 150,000 in 1907, a variation of more than fifteen fold. In one year the population was sometimes more than eight times as large as in the preceding year.

When the Biological Board of Canada undertook in 1925 to compare artificial with natural propagation in the case of the sockeye salmon it was necessary to determine the actual number of fish entering the experimental area. This was done by building a fence across the Vedder River to stop all fish on their upward migration and constructing traps in which to catch them. By this means it was found that when populations were accurately determined the variations were actually greater than those suggested by the commercial catch. In one year (1927) 82,425 adult sockeye entered the Vedder River whereas in another (1932) only 2,259 appeared (Foerster, 1938). In this case the big run was more than thirty-six times as large as the small one.

Such fluctuations in populations, it is admitted, are exceptional. Usually animals maintain more uniform numbers from year to year but accumulating evidence suggests that fluctuations in animal populations in nature are the rule rather than the exception. To understand the reasons for these fluctuations it is necessary to understand how the size of population of animals in nature is determined.

The size of a population of any species is the result of the interaction of two sets of forces often designated as biotic potential and environmental resistance. Biotic potential may be defined as the potential power of reproduction of a species under ideal conditions. This power is always tending to increase the size of a species' population. The increase is resisted by such factors of the environment as food scarcity, predators, disease, and unfavourable meteorological conditions. Such environmental factors together constitute the environmental resistance. Since it is difficult to conceive of the biotic potential, that is the inherent reproductive capacity of a species, as varying except as a result of environmental changes, it may be assumed that all population fluctuations are due to environmental changes.

A striking illustration of the way in which populations react to environmental changes was afforded by Manitoba's muskrat management project. Following the construction of a system of dams, dykes, and canals, as a result of which suitable muskrat habitat was restored in dried up marshes, the muskrat population in one area increased from 1,000 to 200,000 in four years.

The extent to which animal populations can increase when food is unlimited is exemplified by an Australian experience during the First

World War, when 544 tons of mice, thought to represent thirty-two million animals, were caught around stacks of bagged wheat awaiting shipment to Great Britain (Elton, 1942).

Many illustrations of the population potentialities of animals have been cited. Bailey's (1924) figure for *Microtus,* the meadow mouse or vole—one million at the end of one year from a single pair at the beginning, if given ideal conditions—is a striking example of the reproductive powers of a common Canadian mammal. With such figures in mind we are perhaps prepared to be surprised, not that populations fluctuate but that fluctuations are not more violent.

Under natural conditions, on the average, only two offspring from a pair of animals during their lifetime reach maturity. If more than two on the average survived to take the place of the two that produced them, the population of that species would in time fill the earth. On the other hand, if fewer than two survived, on the average, the species would gradually die out. The environment is continually changing, leading to the production and survival of a varying number of young and adults from time to time. This results in variations in the size of populations which, over a period of years, fluctuate about a mean.

When the population of a species attains unusual abundance, it always results from some lessening of the environmental resistance usually through provision of abundant food, or reduction in numbers or absence of predators, or both.

The history of moose on Isle Royale, Lake Superior, affords one example of the type of experience which has been repeated by many kinds of animals under many different conditions. Although there may have been moose on the island in earlier times, their recent history there appears to have begun during the winter of 1912-13 when a few animals made their way to the island over the ice which formed that year between it and the Canadian mainland, thirteen miles away at the nearest point. With plenty of food available and in the absence of hunters, wolves, or other large predators, moose increased so that when Murie (1934) made his survey in 1930 he estimated a population of between one and three thousand. The size of the island is 220 square miles and at that time the vegetation gave many evidences of being over-browsed. Between 1929 and 1935 the moose population on Isle Royale decreased from a high of one to three thousand to about two hundred (Shaler and Krefting, 1946) presumably as a result of the depleted food supply which, in turn, had resulted from the over-population.

An example from the opposite end of the size-scale of mammals was afforded by a California experience with mice. In 1926 a plague of mice involving at least tens of millions and possibly hundreds of millions spread over the country-side from their point of origin in the dry bed of Buena Vista Lake. Here during the previous year or two the multiplication of the mice had been favoured by abundance of food and scarcity of predators. The plentiful food supply was in the form of barley and Kaffir corn left on the ground after harvest. The scarcity of predators was in part due to an intensive poison campaign against coyotes which had been carried on during the previous year or two. Other predators including kit foxes and skunks had been poisoned as well as coyotes. Protection from winged predators was afforded the mice by Kaffir corn stalks from which only the heads had been cut. Finally the mice (nearly all *Mus musculus* but some *Microtus californicus*) left their breeding grounds and spread out over the surrounding farms. The descriptions of the numbers of mice involved are almost unbelievable. In one trench made in the path of the migrating mice an average of between fifteen and sixteen dead mice per linear foot was found on the third day after the trench had been made. This trench was about five miles long, and it was computed that one-half million mice had been killed by eating poisoned grain placed in it. "At night, on the highway . . the illumination from the headlights of one's car revealed hundreds of live mice at any given instant . . . Truly, the number of mice was almost unbelievable, and one who has not seen this or a similar outbreak can scarcely comprehend the vast numbers that can occur in a given area of limited extent. Certainly the numbers were to be reckoned in tens, and possibly in hundreds, of millions."

Sometimes the introduction of an animal into a favourable new environment is followed by spectacular increases in numbers. The increase and spread of rabbits in Australia is a well-known example but we have several closer to home.

The European starling introduced into Central Park, New York City, in 1890 and 1891, arrived in Ontario in 1919 and in about fifteen years, according to L. L. Snyder, became the most abundant bird in Ontario south of Lake Simcoe.

The history of the smelt *(Osmerus mordax)* in the Great Lakes affords another striking example of the attainment of a large population by an introduced species (Dymond, 1944). It was first recorded from Lake Huron in 1925 and first spawning runs in streams of the north shore occurred in 1932. By 1941 there was not a stream, large or small, along all of Georgian Bay that was not visited at spawning time. In some the smelts were so abundant that sufficient were caught to fill steel-bodied gravel dump trucks in half an hour. From two to four tons were taken from one creek in three hours' time on more than one occasion. In this case, as in that of many others when animals become very abundant, a drastic decline in population was experienced. In the latter part of October, 1942, tremendous numbers of dead smelts, described as "a solid white sea of dead smelts," were seen floating on the water in the North Channel. On many parts of the north shore of Manitoulin Island, dead smelts were piled to a depth of at least one and a half feet. As a result, the next spring spawning runs were greatly reduced and in many streams where large runs had occurred in previous years smelts were entirely absent. (Stevenson, 1944.)

Two types of population fluctuation may be recognized—the periodic and the non-periodic. Examples of the latter are afforded by such occurrences as the California mouse plague, and the appearance of moose in Isle Royale and smelts in Lake Huron. In such cases some unusual combination of favourable conditions brings about an exceptional abundance of some species which, when the favourable conditions cease to exist either through the action of the animals themselves or otherwise, declines, often drastically.

PERIODIC FLUCTUATIONS

The populations of many animals undergo periodic fluctuations, that is changes from abundance to scarcity which recur at rather regular intervals. It is to Elton (1924, 1927) that we owe the great development of interest in this phenomenon although Poland (1892), Macfarlane (1905), Seton (1911), and Hewitt (1921) had previously drawn attention to it.

Reference has already been made to the varying hare or snowshoe rabbit (*Lepus americanus*) whose populations have reached peaks of abundance usually at intervals of nine or ten years for at least as long as Hudson's Bay Company records are available (Fig. 2). During the period 1856 to 1934 there were nine occasions on which the numbers of hares reached peak proportions (MacLulich, 1937). On each occasion these peaks were followed by drastic reductions. This represents an average of 9.7 years between maxima. In three cases the period between maxima was 10 years, in two cases it was 9 years, in two others 11 years, and in one 8 years. The ruffed grouse (*Bonasa umbellus*) has a periodic fluctuation in population corresponding closely to that of the varying hare, namely between nine and ten years on the average (Clarke, 1936).

Another remarkable periodic phenomenon connected with animal populations is the arrival of snowy owls in southern Canada and northern United States every four or five years. There is strong circumstantial evidence for the conclusion that the emigration of these owls from their Arctic homeland is associated with the attainment of a population peak following an increase in the numbers of their staple food animals, the lemmings. This conclusion is based on the occurrence of maximum populations of Arctic foxes in the north in the years when snowy owls appear in southern Canada. Lemmings are the staple food of both Arctic foxes and snowy owls. Gross (1931) compares the two occurrences as shown in Table I.

TABLE I

YEARS OF MAXIMUM NUMBERS OF ARCTIC FOXES AND OF SNOWY OWL EMIGRATIONS

The years of maximum numbers of Arctic foxes at Fort Chimo	*Winters of noteworthy appearances of snowy owls in southern Canada and northern United States*
1872	
1876	1876-7
1879	
1882	1882-3
1887	
1890	1889-90
1893	1892 3
1897	1896 7
1901	1901-2
1905	1905-6
1909	
1913	
1917	1917 18
1921	
1926	1926 7
1930	1930 1

Large flights of snowy owls have also appeared in 1934-5, 1937-8, 1941-2, 1945-6. Mr. L. L. Snyder of the Royal Ontario Museum of Zoology reports that estimates of the comparative numbers involved in recent flights are as follows, the number of the 1926-7 flight being represented arbitrarily by the figure 10.

1926-7	10
1930-1	6
1934-5	4
1937-8	2
1941-2	5
1945-6	20+

Following is a list of Canadian animals whose population fluctuations have been shown to be definitely periodic in frequency.

TABLE II

SPECIES OF CANADIAN ANIMALS SHOWING PERIODIC FLUCTUATIONS IN ABUNDANCE

Species	*Usual period*	*Extreme range*	*Authorities*
MAMMALS			
Lepus americanus	9 10	8-11	Poland (1892), Macfarlane (1905) Seton (1911), Hewitt (1921) Elton (1924), MacLulich (1936)
Ondatra zibethica	9 10	?8 12?	Elton and Nicholson (1942a)
Microtus pennsylvanicus	4	?	Hamilton (1937), Banfield (1946)
Lemmus trimucronatus *Dicrostonyx hudsonius*	4		Elton (1924, 1942)
Vulpes fulva	9-10	8 13	Hewitt (1921), Cross (1940)
Alopex lagopus	4	3 6	Hewitt (1921, Elton (1924, 1942)
Martes americanus	9 10	8-11	Hewitt (1921)
Martes pennanti	9-10	8 11	Hewitt (1921)
Mustela vison	9-10	6 12	Hewitt (1921)
Lynx canadensis	9-10	7-12	Hewitt (1921), Elton (1924, 1933b) Elton and Nicholson (1942b)
BIRDS			
Accipiter gentilis	9 10		Speirs (1929)
Buteo lagopus	4	3 5	Speirs (1929)
Bonasa umbellus	9 10		Criddle (1930), Clarke (1936)
Lagopus lagopus	9-10	9 12	Clarke (1936)
Tympanuchus cupido	cyclic		Leopold (1931)
Pedioecetes phasianellus	9-10		Criddle (1930), Snyder (1935)
Bubo virginianus	9 11		Speirs (1939)
Nyctea scandiaca	4	(2?) 3-5 (6?)	Gross (1931, 1937) Speirs (1939), Snyder (1943)
Lanius excubitor	4	(3?) 4-5 (6?)	Davis (1937), Speirs (1939)
Pinicola enucleator	5 6		Speirs (1939)
FISH			
Salmo salar	9-10		Huntsman (1931) Griswold (1929) Phelps and Belding (1931)
Onçorhynchus nerka	4 in Fraser River watershed		Mooney (1861) Babcock (1902) Foerster (1929)
Oncorhynchus gorbuscha	2		Pritchard (1937)

Although the periodicity of *Microtus* has not been established in Canada, Banfield (1947) has recorded evidence that strongly suggests a cycle. An unusually high peak of abundance of these mice occurred in southern Ontario in 1936 and farther north in 1937 (Whelan, 1939). Banfield's observations at Toronto suggested peaks in 1935, 1940, and 1945. A historic "plague" of voles occurred in Nova Scotia in 1815 (Patterson, 1887) during which the mice were so numerous that "over acres and acres they left not a stalk standing nor a grain of wheat to reward the labour of the farmers. . . . Farmers had to sell off a great part of their stock at low prices."

CAUSES OF NON-PERIODIC FLUCTUATIONS

Non-periodic fluctuations in populations are probably to be accounted for through the action of some unusually favourable or unfavourable environmental condition or combination of conditions. The combination of conditions favouring the increase of mice which led to the California mouse plague has already been discussed.

The occurrence of abundance of a particular type of plankton at the time and place when and where newly hatched fish occur has been suggested as the explanation for the origin of unusually successful year-classes which have been shown to characterize many species of food fishes in European waters (Hjort, 1926).

The clue to the explanation of the wide but irregular fluctuations in the populations of many marine fishes was found by Hjort (1914) in the occurrence of striking differences in the strength of different year-classes of herring in Norwegian waters. He found for instance that the herrings spawned in 1904 were so abundant that they dominated the Norwegian herring fishery for a long period of years. Other good years were 1913 and 1918 while intervening years were poor. The same phenomenon of unusually successful and also unusually unsuccessful year-classes has been found to occur with many of the important food fishes in European waters. Sometimes the members of one year-class will be fifty or sixty times as numerous as those of another. That similar conditions occur in Canadian waters has been shown by the work of Hart (1933, 1943), Tester (MSS.), and others in connection with researches of the Fisheries Research Board of Canada, as will be outlined later.

Many of the wide fluctuations between abundance and relative scarcity which occur in Great Lakes fisheries are probably to be explained on the basis of great differences in the strength of year-classes. For instance the present abundance of lake herrings in Lake Erie is known to consist largely of a single year-class. When the members of this year-class have been caught or have died off, lake herring fishing will again be poor unless another successful brood year appears.

Some light was thrown on the question as to the stage at which losses occur in an anadromous fish, the pink salmon *(Oncorhynchus gorbuscha)*, by the results of studies carried out on McClinton Creek, Queen Charlotte Islands, by Pritchard (1938) under the auspices of the Fisheries Research Board. In connection with these studies, a fence and traps were constructed by means of which all adults entering and all young leaving the experimental stream were trapped, for counting. Table III gives the number of adults and young respectively entering and leaving during each year of the experiment. There is in this case a single population concerned, the seaward migrants of one year producing the adults of the following year. We therefore have information on the size of the population of a year-class at three stages, as we know (1) the number of parents, (2) the number of resulting seaward migrants, and (3) the number of resulting adults. Table III gives this information for each of the years during which the experiment was carried on. It indicates that during the period covered there was a fifteen fold variation in the size of the run.

TABLE III

NUMBER OF PINK SALMON AT THREE STAGES IN THE LIFE HISTORY OF A YEAR-CLASS (Fry from 1930 spawning produce 1932 adults, etc.)

	Adults	*Seaward migrating fry*	*Per cent of eggs producing seaward migrating fry*	*Percent of fry producing adults*
1930	66,153	5,384,000	10.6	0.29
1932	15,600	2,300,000	17.3	6.74
1934	155,196	12,600,000	9.0	0.42
1936	52,312	3,675,000	6.9	0.29
1938	10,577	2,020,000	23.8	1.75
1940	35,525	5,060,000	19.0	0.73
1942	36,893			

While there was a wide difference in the percentage of eggs which produced seaward migrants, this factor is apparently not the most important one so far as the number of adults returning to the stream is concerned. This is illustrated by the fact that roughly 2¼ million seaward migrants in 1932 (the second smallest number of fry found during the seven cycles) produced the largest population of adults, namely 155,196, whereas more than 12½ million seaward migrants in 1934 produced only 52,312 adults in 1936. It is of course not certain that all of the adult progeny from a given stream return to it when they mature or that all adults entering a stream were produced in it. It is obvious however that the size of the run of adults to the experimental stream bore little relationship to the number of young of the year-class to which they belonged. This lack of correlation was due more to the extent of the loss from seaward migrant to returning adult than to the loss from egg to seaward migrant.

In an effort to secure information on the extent to which fish return to their natal stream, a large number of fry were distinctively marked in several years by removal of certain fins and rewards were offered for the detection of fish so marked in other parts of British Columbia and in Alaska. In all cases most of the marked adults that were identified were found in McClinton Creek. Some were taken by commercial fishermen in areas from which it is reasonable to expect they might have returned to the home stream. A few were captured in areas from which return was quite improbable. That is, of 108,000 progeny of the 1932 spawning which were marked, 3,285 were subsequently recovered, 2,950 of them in the parent stream: of the others, only 7 were taken outside the Queen Charlotte Islands area.

Mortality from seaward migrant to the entrance of the adult into the natal stream will include both natural and fishing mortality. In the McClinton experiments it was impossible to estimate variation in fishing mortality from year to year. The results of the experiment are therefore not conclusive but they strongly suggest great differences in natural mortality in the sea stage of the life history of the pink salmon.

CAUSES OF PERIODICITIES IN POPULATION FLUCTUATIONS

Several explanations of the regularity which characterizes many fluctuations in animal populations have been suggested. Lotka (1920) and Volterra (1926) on the basis of mathematical theories have shown how oscillations are inherent in some population systems and Gause (1934) has shown that under ideal experimental conditions some of their mathematical equations seem to be realized. However the value of these mathematical theories in the study of populations in nature is still in doubt.

The belief that there was some sort of relationship between the sun-spot periodicities and the periodicities of hares and grouse was rather widely held for a time (Elton, 1924; De Lury, 1930; Wing, 1935). MacLulich (1937) is generally believed to have disposed of this theory so far as the hare is concerned when he showed that the animal and solar cycles are of different lengths and that therefore, although there are recurring periods during which the animal peaks correspond more or less closely to the sun-spot maxima, these are followed by periods when animal maxima are in phase with sun-spot minima. Although MacLulich's arguments have convinced many (Elton and Nicholson, 1942a) the sun-spot theory appears still to fascinate others (Clements and Shelford, 1939). It is the belief of the present author that the explanation of the regular periodic fluctuations in some animal populations can be found without calling in the aid of extra-mundane factors.

Leaving aside the Atlantic and Pacific salmons, which require a separate discussion, the animals whose populations have been shown to present regular periodic fluctuations belong to two categories: (1) predators whose periodic fluctuations obviously depend on the periodic fluctuations of their prey and (2) rodents or other essentially plant-eating animals whose periodicities have not been shown to depend on any periodic factor in their environment.

The dependence of the periodicities of a number of predatory mammals and birds on the periodic fluctuations in the populations of their prey is obvious. The dependence of the Canada lynx *(Lynx canadensis)* nine- to ten-year periodicity on the corresponding periodicity in the hare *(Lepus americanus)* is generally admitted. To a surprising extent the food of the lynx is largely restricted to hares. The correspondence between the curves representing the number of lynx and the number of hare skins from Canada sold in London by the Hudson's Bay Company is remarkably close. As is to be expected in the case of a predator the curves representing its population increase are often one year to the right, i.e. later, than the corresponding hare curves.

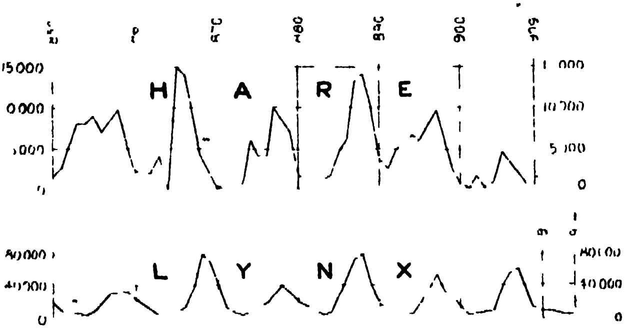


FIGURE 2. —Correlation between periodic fluctuations of hare and lynx populations as represented by Hudson's Bay Company fur returns, 1850-1909. (Based on Hewitt, 1921.)

The fact that coloured foxes *(Vulpes fulva)* and the marten have a four-year cycle in the Arctic and a nine- to ten-year one farther south is strong evidence for the belief that the length of the cycle in predatory forms depends on the periodicity of the prey species. The food of fox *(Vulpes fulva)*, marten *(Martes americana)*, fisher *(Martes pennanti)*, and mink *(Mustela vison)* is not so restricted to hare as it is in the case of the lynx. It should be remembered, however, that the fur statistics used to establish the periodicities of these animals are for northern Canada where the variety of prey is much more restricted than it is farther south. One indication that marten numbers are influenced by hares is afforded by the fur statistics of Douglas for British Columbia as reported by Cowan (1938). On the mainland marten peaks occurred in 1826, 1836, 1845, and 1853, whereas on Vancouver Island, where there are no hares, marten peaks occurred in 1842, 1852, 1855, and 1857. It is generally accepted, I believe, that all of these predatory mammals prey more or less extensively on hares when the latter are available. The same is true of two large predatory birds, the goshawk (*Accipiter gentilis*) and horned owl (*Bubo virginianus*), whose periodicities correspond with that of the hare. No one, I believe, questions the direct connection between the four-year periodicity of the Arctic fox (*Alopex lagopus*) and snowy owl (*Nyctea scandiaca*) and the corresponding periodicity of the lemming (*Lemmus trimucronatus* and *Dicrostonyx hudsonius*). The periodicity of the rough-legged hawk probably also depends on the lemming periodicity.

In the case of carnivorous mammals and birds, it is the size of the population of their prey animals that determines the size of their populations and not *vice versa*. Errington (1946) in his exhaustive review of predation and vertebrate populations concludes that "regardless of the countless individuals or the large percentage of the population that may annually be killed by predators, predation looks ineffective as a limiting factor to the extent that intraspecific, self-limiting mechanisms basically determine the population levels maintained by the prey." The fact that "among the mammals and birds, the numbers of those tolerant of crowding appear most influenced by predation" is possibly significant in connection with the problem of periodicities as we shall see later.

We therefore narrow down our problem of accounting for the regularity in the population fluctuations among Canadian birds and mammals to that of accounting for the periodicities of relatively few, including the varying hare, muskrat, meadow mouse or vole, lemmings, ruffed grouse, etc.

The following characteristics of these animals and of their periodicities appear to be significant.

1. The variation in the length of cycle from time to time within the same species and the rather wide difference in time at which peak populations are reached in different parts of the country.

2. The lack of uniformity in the average length of the cycle in different species—four years in lemmings and *Microtus*, six in pine grosbeak and nine or ten in the hare, the muskrat, and the grouse and its relatives.

3. The fact that these are all Arctic or boreal animals.

4. The fact that the mammals are all rodents and the birds with the exception of the pine grosbeak all grouse or relatives of the grouse.

Although the periodicity of the hare must be recognized as remarkably regular, it is easy to over-emphasize the regularity. As MacLulich

(1937) has pointed out, the Hudson's Bay Company records on which these figures are based are for a limited area of Canada, namely the region surrounding Hudson Bay. When the whole of Canada is considered, the peaks do not coincide but occur in different years in different areas. MacLulich found that the onset of diminution of numbers, which is more definitely determinable than the attainment of maximum numbers, occurred in south-eastern Ontario in 1932, in central Ontario in 1933, from Temiskaming to Kenora in 1934, in the northern part of the clay belt in 1935, on the coast of James Bay in 1934 and in the southern part of the Patricia District in 1933.

Clarke's (1936) work on the ruffed grouse also showed that the periodicity of this species when studied carefully is not nearly so regular as might be supposed. Clarke found the first evidence of diminution of numbers in Canada in the nineteen-thirties occurred in 1930 in Nova Scotia. In 1932 all the reports of decrease came either from the east coast or the north-west while the first evidence of cyclical decrease in Ontario came in 1933. The south-western part of the province, which was the first to suffer a decrease in hares, was the last to record a decrease in grouse. When the conditions of grouse abundance in areas of Canada outside Ontario were considered, differences in the year of commencement of the diminution in numbers were found to extend over a number of years equal to at least half of the periodicity of the cycle. Clarke considered it possible that grouse might be found in any given stage of the cycle in some part or other of North America in any given year.

Although the periodicity of the fox is secondary, depending on that of the hare, perhaps influenced by fluctuating abundance of mice, a study of its periodicity by Cross (1940) contributes to an understanding of the extent of variation in the length of the period of population fluctuations and of the variation in the time at which peaks are attained in different areas. This study shows that in the case of the red fox, periodic fluctuations in numbers are not province-wide but are definitely regional phenomena. Cross's records covered a period of twenty years, 1916-35, and included records from thirty-four trading posts of the Hudson's Bay Company in northern Ontario and of more than 100,000 fox pelts. He demonstrated that a large proportion of the posts reached a maximum catch of foxes in years other than the peak years. For instance the maximum for the whole province was reached in 1917, in 1926, and in 1936 yet in 1917 only 52 per cent of the individual posts reached their maximum; in 1926 the corresponding number was 50 per cent while in 1936 it was only 30 per cent. There was also a wide variation in the interval between peaks at different posts. Between the first and second of the three peaks discussed the interval was:

8 years in 14 per cent of the posts
9 years in 24 per cent
10 years in 43 per cent
11 years in 14 per cent
13 years in 5 per cent

Table II indicates the extent of variation (extreme range) in the length of cycles in different species.

It has long been recognized that periodic fluctuations in animal populations are virtually confined to the northern part of the northern hemisphere and are especially characteristic of the Arctic, not only in America but also in Europe and Asia where the same species—lemmings, snowy owls, and Arctic foxes in America and lemmings and snowy owls in the Old World—undergo violent periodic fluctuations. The phenomenon is still pronounced in the north temperate region but disappears southward. Cowan (1938) on the basis of records in the note-book of James Douglas reports that in the southern part of British Columbia rabbits (hares?) never underwent the extreme fluctuations in numbers characteristic of the north. This is reflected in the fact that lynx numbers increased a hundred fold from lowest ebb to highest peak in the northern part of the province, through an intermediate increase of fifty fold in the intermediate latitude of Langley to a relatively stable population (an increase of but twenty fold) at Fort Vancouver three hundred miles farther south.

Finally, the fact that the "key industry" animals whose fluctuations in numbers are periodic are all rodents or grouse-like birds (with the exception of the pine grosbeak) is undoubtedly significant.

Briefly the theory here offered to account for the periodicity in the fluctuations of the populations of lemmings, hares, voles, grouse, etc., is that the biotic potential of these animals is definitely greater than the normal resistance of the environment in which they live. In other words they are endowed with too high a reproductive capacity and it is the monotony or relative uniformity of the Arctic and far northern environment that accounts for the regularity with which they increase to the unstable level of numbers which brings about the periodic decline.

It should be emphasized that it is the regularity of the events producing a decline in populations that must be accounted for. Any small population of an animal placed in a suitable environment will normally increase in size. Either one of two occurrences normally brings the increase of population of the animals under discussion to an end and produces a decline. One of these is emigration, the other is disease.

EMIGRATION AS A FACTOR IN POPULATION FLUCTUATIONS

Emigration as a means of population reduction occurred in the case of the California mouse plague already referred to, but perhaps the most famous example is afforded by the lemmings of Scandinavia. Elton's (1942) description gives a picture of these emigrations. "In the big years lemmings begin to travel down the mountain sides and valleys through the tree belt . . . out over the lowlands, crossing fields and lakes and rivers and passing through busy towns, until those that survive reach the coast. Here they swim out . . . many drown . . . and are cast ashore in drifts. . . . After a year or two, and mostly much sooner, all of them are dead. There remain a number of their depleted populations on the high hills, as nuclei of further waves of lemmings."

There are comparatively few records of lemming emigrations in Arctic America. Anderson (1937) suggests that the lack of such records is due partly to the fact that there are fewer observers in Arctic Canada and partly to the comparative flatness of the Canadian Arctic generally, which does not give opportunities for concentration of the animals in settled regions at the base of mountains as in Norway, Sweden, Finland, and Russia. The following three records, however, attest the fact that migrations do occur in our Arctic. Rae (1890) reports that when travelling in June, 1851, southward from the Arctic coast along the west bank of the Coppermine River and north of the Arctic circle he met with thousands of lemmings speeding northward. A big migration of *Lemmus* at Point Barrow, Alaska, in 1888 is recorded by Allen (1903) and Anderson (1935) says that Captain Berthé witnessed a big migration of *Lemmus trimucronatus* in May, 1926, at Baker Lake.

Snyder (1935) has recorded a spectacular emigration of sharp-tailed grouse *(Pedioecetes phasianellus)*. In the autumn of 1932, grouse of this species arrived in numbers in many sections of northern Ontario where they are normally absent or not very common. They were observed near the end of October passing over Moosonee in vast flocks that reminded the observer "of the stories he had heard of the passenger pigeon in years gone by." This unusual migration continued at approximately the same rate for about three weeks. While the evidence is not as conclusive as could be desired, largely owing to the lack of observers in the area from which the grouse originated, there is reason to believe that this emigration followed a peak of numbers in the area concerned. A similar emigration had occurred in 1896.

Emigration, as already noted, occurs every four years on the average in the case of the snowy owl, presumably at times of peak abundance. Although less spectacular, periodic emigrations of goshawks and great horned owls also occur.

Other animals which show periodic fluctuations in population but do not normally emigrate, occasionally do emigrate. That mass movements of varying hares sometimes occur is attested by Cox (1936) who witnessed such a movement at the time of hare abundance in northern Minnesota. As is usual in such cases, the animals all moved in one direction. The numbers involved are suggested by the fact that at the farther edge of a large lake which the hares crossed in their travels "thousands" had fallen victims of hawks and owls that had evidently gathered there to prey on them.

Elton (1942) reports that the Arctic fox occasionally moves southward in numbers. In 1922 they reached as far as the Canadian Labrador. In the spring of 1923 one was shot on the coast of Cape Breton Island. Emigrations occasionally occur also among lynx, marten, and coloured foxes.

The phenomenon is also known among animals whose populations are not cyclic; for example, Seton (1928) records the movements of "immense numbers" of gray squirrels *(Sciurus carolinensis)*.

DISEASE AS A FACTOR IN POPULATION FLUCTUATIONS

That disease is commonly responsible for the decline in the numbers of animals which have reached a high level of population has long been recognized. Darwin (1859) observed that "when a species, owing to highly favourable circumstances, increases inordinately in numbers in a small tract, epidemics—at least, this seems generally to occur with our game animals—often ensue."

Even in the case of animals where shortage of food resulting from over-population leads to population reduction, this is often brought about through the action of parasites and disease rather than through actual starvation. Malnutrition as well as crowding favours the spread of parasites and disease organisms.

That disease is the factor responsible for the rapid decline in the numbers of hares following the attainment of their population maxima is well established. From MacLulich's (1937) excellent summary of the evidence we learn that a variety of diseases are responsible and that the particular disease or combination of diseases varies from cycle to cycle and from place to place during the same cycle. He found that a blood-sucking stomach worm (*Obeliscoides cuniculi*) caused an epidemic at Smoky Falls, Ontario, in 1935.

Green (1935) once suggested that tularaemia might be responsible for the periodic decline in hare population (see also Huntington, 1932) and later (1939) suggested a "shock" disease as the explanation. MacLulich showed that while tularaemia occurred among varying hares in widely separated parts of Ontario it was not present even at the time of their dying-off in any large percentage of the population.

Clarke (1936) found disease to be the immediate cause of the periodic decrease in ruffed grouse populations. Although several parasites and diseases were found, the only organism significantly associated with the diminution of grouse numbers during the cycle which he investigated was a blood protozoon, *Leucocytozoon bonasae*.

Elton (1935b) has described the periodic epidemic among *Microtus agrestis* in England. A protozoan infection of the brain due to *Toxoplasma* has been shown to be the cause. An epidemic among American voles has been recorded by Hamilton (1937). At the time of the 1926-7 California mouse plague already mentioned, Wayson (1927) isolated the causative bacillus of mouse septicemia or swine erysipelas (sometimes considered identical) from morbid microtines involved in the outbreak. Evidences of disease among voles are recorded in connection with the famous Nova Scotia plague of mice in 1815. It is recorded (Patterson, 1887) that in the autumn the mice "became languid, scarcely able to crawl. One could trample them under his feet and finally they died in hundreds, so that they could be gathered in heaps."

The relation of population density to the spread of disease was pointed out by Ross (1911) in connection with the control of malaria. He formulated mathematically an equation of the struggle for existence between malaria plasmodium and man with participation of the mosquito. Disease is always present but reaches plague proportions only after the host has passed a certain threshold of numbers. Fluctuations in weather and other ecological conditions may modify the length of time needed to reach the required level of numbers. The fundamental periodicity, however, is probably determined by the time required to reach the threshold of numbers necessary for disease spread.

EMIGRATION PHENOMENA

There is strong evidence that emigration, like disease, is the result of a certain rather high density of population. The most obvious suggestion as to the initiating factor in emigration is that it follows exhaustion of the food supply. However, observations have usually failed to find evidence of lack of food in the case of emigrants. Snyder (1935) reports that the specimens of sharp-tailed grouse collected during their 1932 flight were in good physical condition. Speirs (1939) quotes C. E. Hope of the Royal Ontario Museum of Zoology, who has examined a large number of goshawks and horned owls collected during their periodic immigration into southern Ontario, as stating that there is no evidence from the birds' condition to indicate that lack of food caused them to come south. Similarly the snowy owls which periodically reach southern Ontario have never been found to show signs of malnutrition.

Uvarov (1921, 1928) has thrown considerable light on the factors involved in the spectacular emigrations which have characterized the populations of locusts in many parts of the world. His studies show conclusively that food shortage plays no part in initiating locust emigrations. The insects leave their breeding grounds while food is still plentiful and pass through and over areas of abundant food on their flights. On the other hand, the phase during which the locusts undertake mass movements is produced as a result of the crowding of a large number of individuals in a limited space.

The insects concerned exist in two quite distinct phases, a solitary phase and a gregarious phase, which differ not only in behaviour but also in morphological and colour characteristics to such an extent that they have been regarded as different species. The two phases are connected by a transitional phase of intermediate characteristics. Uvarov believes that either of the extreme phases can be transformed into the other but Allee (1931) does not think it has been clearly proved that the transformations from one phase to another do actually take place on a large scale and to a convincing degree. While the solitary phase consists of isolated individuals, the gregarious or swarming phase forms dense emigrating swarms. It is stated that, once the development of the swarming phase has begun as a result of crowding, this process can only increase in intensity because the bands once formed unavoidably grow during their wanderings owing to fusion with other bands. Only sufficiently large swarms emigrate.

Uvarov's description of emigrating locusts reminds us of the accounts of emigrating lemmings and other animals. "The relentless march of such masses of hoppers, regardless of obstacles, creates an impression of some dark purpose, of a movement towards an objective, and has led to numerous more or less fantastic explanations of the cause of movement based on the imagination of the observer and not on the facts. Obstacles to the moving bands, in fact, do not seem to exist; they pass over hillocks and boulders; holes and ditches in the ground are filled with the bodies of the first coming hoppers, and their comrades pass over them as if over level ground. . . . Across canals and streams the bands swim to the other side and continue their march."

The emigratory behaviour of locusts therefore has many features in common with that of lemmings and in many respects in both animals is in striking contrast to the behaviour shown in their breeding territories. Similar difference in behaviour is recorded by Kennicott as quoted by Seton (1928) in the case of the gray squirrel *(Sciurus carolinensis)* which formerly undertook mass movements. Kennicott says: "Immense numbers congregate in autumn and move off together, continuing their progress in the same general direction, whatever it may be . . . not even turning aside for large streams. Ordinarily averse to entering the water, they now take to it boldly, and though swimming with difficulty, manage to cross broad rivers like the Niagara and the Ohio, though many are drowned in the attempt." During one of these migrations "innumerable squirrels" are said to have swum across the river near Niagara and landed near Buffalo.

Seton was unable to find any records of these mass movements of squirrels after 1870 but Hamilton (1939) records them in Connecticut and New York in 1933 and 1935. The fact that such movements are rare now as compared with former times suggests that they were a feature of the squirrels' behaviour before their populations were reduced through the destruction of the hardwood forests which provided them with such abundant food in earlier times.

Whether the "crazy" flight of the ruffed grouse is another manifestation of abnormal behaviour associated with emigration resulting from crowding is not clear. Two characteristics suggest that it is. Although grouse do not normally form large flocks, Clarke (1936) quotes an observer as saying that he had seen the grouse cross the Ottawa River "in large numbers." The other circumstance which suggests a similarity of these "crazy" flights to certain characteristics of the mass emigration often observed among other species is their abnormal nature, indicated by their popular description as "crazy."

The evidence therefore suggests that emigration may not be due to food shortage but is rather a reflection of abnormal behaviour patterns resulting from crowding.

Allee (1931) in his book on *Animal Aggregations* shows that, while under many conditions there are distinctly beneficial results from crowding, when it is too great it is harmful, especially for poorly integrated groups of animals.

The intolerance to crowding shown by certain species may be related to the phenomenon of territoriality (Nice, 1933). If this is the case, it might explain the fact that some animals when crowded emigrate before food is exhausted while others, as for example deer, remain in crowded areas until the food supply is so reduced that they succumb to starvation or diseases favoured by malnutrition. In the case of dense populations there appears to be a race between disease, emigration, and starvation as to which is to reduce the numbers, with the decision determined largely by the reaction of the species to crowding.

The reaction of animals under crowded conditions is sometimes referred to as arising from intraspecific tension or population pressure. The latter expression may have been suggested by a comparison of the outward movement of the animals from their home ranges to an explosion. The fact that the latest emigration of snowy owls, which was the largest on record, carried the birds the farthest indicates that the comparison of emigration to an explosion is, in that respect at least, appropriate.

That the whole phenomenon is far from simple is indicated by the fact that in spite of the emigratory animals acting as if they were trying to get away from crowded conditions, they sometimes move in vast flocks. The factors involved in the initiation and continuance of emigratory movements are complex and will provide fascinating problems for students of animal behaviour.

PERIODICITY IN ATTAINMENT OF HIGH POPULATION LEVELS

Whether the factor which at the end of a fairly definite number of years brings about the population decline is disease or emigration, it appears to be initiated by conditions arising from the attainment of a rather high level of population density. Why is it that in the case of the animals under discussion—lemmings, voles, hares, grouse, etc.—this critical level of numbers is always reached after roughly the same number of years? A species placed in a perfect environment without variation in any of its components would always reach the same level of numbers in the same period of time, while variation in the action of any environmental factor would produce a variation in the length of time required to reach a given level of numbers unless compensated for by variation in some other factor. These species will show the more regularity in the period required to reach a certain level of numbers the less they are affected by environmental changes.

While the Arctic environment cannot be described as uniform, it is in a number of respects much more nearly uniform than environmental conditions farther south. This is true for instance in the matter of predators. The Arctic lemming has comparatively few predators, these including the snowy owl, rough-legged hawk, gyrfalcon, and Arctic fox, whereas a rodent of comparable size in the austral region of North America has a host of predators, such as red and gray foxes, coyote, raccoon, opossum, spotted and striped skunks, weasel, mink, badger, shrews, marsh, red-shouldered, and red-tailed hawks, barn and barred owls, as well as several species of snakes including a number of species each of king snakes, milk snakes, gopher snakes, rat snakes, and rattlesnakes. Moreover there are in the environment of the austral rodents many buffer animals which share with them the predator pressure of the carnivores including meadow mice, white-footed mice, pine mice, pocket mice, harvest mice, jumping mice as well as many somewhat larger forms such as chipmunks, cotton rats, kangaroo rats, pocket rats, and wood rats. The effect of "buffers" on the populations of other animals has been pointed out by Errington (1945). So far as the predator part of the lemming's environment is concerned, it must exert a fairly uniform pressure, the Arctic foxes and snowy owls increasing in fairly direct ratio to the lemmings since their populations are dependent on that of the lemmings. In the case of austral rodents, the populations of their predators are not so directly dependent on that of a single species but on the populations of a variety of prey. Similarly the predator pressure is not exerted on a single species but is buffered by a variety of other prey animals. There is therefore not the same uniformity of relationship between predator and prey in the south as in the north.

It is unlikely that the character of the environment is alone responsible for the regularity with which the populations of these animals reach the critical density which initiates the decline. The reproductive capacity and habits of the animals probably also contribute to the cumulative increase which characterizes their normal population dynamics It is undoubtedly significant that the only Canadian mice which have been shown to exhibit periodic fluctuations in population are so closely related genetically. Their habit of burrowing beneath the snow tends no doubt to insulate them from the effects of some of the variations in climatic conditions.

The suggestion that the character of the animal as well as the character of the environment has a bearing on the cyclical nature of their population histories is supported by the fact that the smaller animals with the higher biotic potential have the shorter cycles. Lemmings and *Microtus* attain the critical population level in four years on the average as compared with nine or ten in the case of hares, muskrats, and grouse.

In contrast to the theory which explains the regular periodicity in population changes as the result of something regularly periodic in the environment the theory here offered would explain the variations in the length of the cycles—two to five years in lemmings and eight to thirteen in hares—as due to variations in the environment and the basic periodicity as due to the essential stability of the environment of animals of high biotic potential whose habits tend to insulate them from extreme climatic conditions.

PERIODICITY IN SALMON POPULATIONS

A nine- to ten-year periodicity in the catch of Atlantic salmon was discovered almost simultaneously by Griswold (1929), Phelps and Belding (1931), and Huntsman (1931, 1937, 1938, 1941). Griswold's discovery was based on a study of the records of the Cascapedia and Restigouche Salmon Clubs and of the fishery statistics of New Brunswick and Quebec. Phelps and Belding corroborated Griswold's findings for the records of the Restigouche Club, finding a periodicity of nine or ten years extending back to 1879. Huntsman used the detailed fishery statistics of the Maritime Provinces which extend back to 1869.

Huntsman concluded that the periodicity in salmon was due to periodicity in rainfall. According to this explanation low water in streams caused by low rainfall exposes the large parr, on which kingfishers and mergansers feed, to increased destruction by these birds. Huntsman further argued that in addition to causing a lowered abundance of one year-class, low water favoured the survival of larger numbers of succeeding year-classes whose young are in the river at the same time, through decrease of cannibalism by the large parr on the younger and smaller salmon. Reduction of the numbers of fish-eating birds has been shown by White (1939) to have more than doubled the number of young Atlantic salmon surviving to the descending smolt stage. That destruction of fish predators increases the survival of young fish was also indicated by the experiments of Foerster and Ricker (1941). In an experiment extending over three years and involving the removal of an estimated 85 per cent of the squawfish, 40 per cent of the trout, and 60 per cent of the char, the rate of survival from egg to seaward migrant was increased three and a third times.

The conclusion that predators may sufficiently affect the size of populations as to bring about periodic fluctuations in fish is contrary to the generally accepted conclusions as to the effect of predators on periodicities among terrestrial animals. Errington in his exhaustive survey of predation and vertebrate populations (1946) concludes that there is more evidence of a significant effect of predators on fish populations than in the case of many other animals.

The four-year periodicity of the Fraser River sockeye salmon appears to be a characteristic of the population of that river system rather than of the sockeye salmon itself. No other sockeye population has a four-year cycle of abundance. The fact that the Fraser River sockeye is predominantly a four-year fish is probably in some way at the basis of its four-year periodicity in abundance. A large year-class once established tends to reproduce itself since the great majority of the Fraser River fish spawn in their fourth year. The Cultus Lake researches of Foerster (1938) under the Fisheries Research Board of Canada showed that the percentage of eggs producing seaward migrants was not much higher in small runs than it was in large ones. During three years (1925, 1927, 1930) of the Cultus Lake experiments comparing the relative efficiencies of natural and artificial propagation, the fish were allowed to reproduce naturally, and the results were as follows:

Year	*Number of adults*	*Per cent females*	*Eggs available for deposition*	*Seaward migrants*	*Percentages producing seaward migrants*
1925	5,423	73	17,470,000	197,700	1.13
1927	82,425	70	250,000,000	2,641,400	1.06
1930	10,395	53	24,900,000	801,300	3.23

The percentage of eggs producing seaward migrants was perhaps not significantly different when 250 million eggs were available for disposition and when the number was only seventeen million. Whether the discrepancy in the sex ratio is responsible for some of the variation in the success of reproduction cannot be decided on the basis of the experiments. It may be significant that the best reproduction resulted when the sexes were approximately equal.

Whether the big sockeye run in the Fraser every fourth year is due entirely to the reproduction of an exceptionally large year-class or whether the big run in some way interferes with the increase in population of the following three years is still a matter for speculation. The fact that in many other species of animals the attainment of dense populations makes the maintenance of such populations impossible suggests that the operation of the same principle be investigated in the case of the Fraser River sockeye.

Two possible ways in which a big year-class may interfere with the production of succeeding year-classes are, first, through older fish preying on younger ones and, second, through removal of some essential nutrient such as phosphorus from the water of the lake in which the young spend a year.

When the adults die after spawning, the disintegration of their bodies must add various nutrient materials, including phosphorus, to the lake through which they pass on their way to the tributary stream in which they spawn. Such nutrients support a growth of plankton which is eaten either directly or indirectly by the young sockeye during the year or more they spend in the lake. A large population of young might lock up in their bodies a considerable proportion of certain nutrients until they in turn die and disintegrate after spawning. Russell (1930 and other papers in the same series) found that a serious decline in the numbers of young fish in the English Channel was correlated with a marked change in the amount of phosphate in the waters. It therefore seems possible that the periodicity in sockeye abundance may be correlated with a periodicity in the supply of some element such as phosphorus which is locked up in the fish.

Another striking periodicity occurs in the case of the pink salmon (*Oncorhynchus gorbuscha*) whose spawning runs almost always occur only every other year. In northern British Columbia the runs are generally in the even-numbered years whereas farther south they are in the odd-numbered years. This species matures in its second year, so that fish which spawned in 1946 were the progeny of fish which spawned in 1944. The fact that runs usually occur only every other year means that there is only a single population which reproduces itself every other year. In explaining the two-year periodicity it is therefore necessary to look for an explanation of the absence of an entire year-class and its progeny through a long period of years.

Attempts of the Fisheries Research Board of Canada to establish a run in McClinton Creek, Queen Charlotte Islands, by transferring to it eggs and young derived from a population which occurred in another part of the islands in the off-years of the McClinton Creek run, failed. The attempts were repeated six times between 1930 and 1942. The reason for the failure of the eggs and young from another population planted in McClinton Creek to produce adults which returned to that creek was not discovered. By distinctively marking the young by the removal of certain fins it was known that none returned to the original stream from which the eggs were derived. Only a very few were taken in commercial fishing operations in spite of the fact that rewards were offered for information supported by the appropriate fin scars of adult fish taken in the fishery. Either the young failed to survive or the adults went to unfished parts of the ocean from which they failed to return to either the original or foster home streams.

The suggestion that a run of pink salmon in one year in some way interferes with the establishment of a population in the following year is strengthened by the fact that when efforts were made to establish pink salmon in some streams in Maine, the fish apparently survived only every other year (Huntsman and Dymond, 1940). Dr. Huntsman has suggested that one year-class eliminates the following one by preying on its young.

IMPORTANCE OF POPULATION STUDIES

Problems presented by fluctuations in animal populations are important for both practical and scientific reasons. In fisheries and wildlife management we are concerned basically with the size of populations. If a species is useful we are usually concerned with increasing its numbers; if it is destructive to our interests our problem is to reduce its numbers.

Before we reach the stage at which we can control an animal's numbers we may be able to predict the approximate size of its populations. Such ability is often of considerable economic value, for instance in planning for the handling of the expected crop in the fur trade or in fisheries. Also, in cases where control is impossible it will often be of economic value to understand the factors producing the result; for instance, the expenditure of funds to increase the population of an animal in a mistaken belief as to the cause of its scarcity may thus be prevented.

The application of a knowledge of cycles to conservation has been discussed by Butler (1942). He estimates that by appropriate conservation measures the fisher catch could be doubled, the lynx catch tripled, and the marten catch increased five times.

Elton on several occasions (1924, 1930, 1942) has emphasized the importance of periodic fluctuations in animal numbers in determining the course of evolution. When the population is at a maximum, selection takes place according to the ability to resist disease and to obtain mates and raise young successfully. At the minimum the animals are selected for their ability to avoid enemies, find mates, and resist unfavourable environmental conditions. Thus selection affects different attributes at the two stages of the population cycle. At the time of the increase in population a mutation having little or no adaptive significance may have an opportunity to become established since there is then relatively little struggle for existence. Elton has enlarged on this subject in a little book entitled *Animal Ecology and Evolution.*

Since epidemics of disease often result when animals reach high levels of population density, the relation of fluctuations in animal populations to public health must be considered. The fact that plague, tularaemia, typhus, relapsing fever, and several other diseases are associated with various rodents is sufficient to emphasize the importance of animal population studies to epidemiology. Cameron (1938) has also pointed out the relation of parasites of wild animals to the health of domestic animals. The comparatively recent discovery by Wells (1937) that a form of tuberculosis occurs in voles in England suggests the possibilities of research in this hitherto largely neglected field.

Many of the principles discovered in studies of the populations of the lower animals will also find application in human affairs. As Pearl (1937) repeatedly emphasized, the biological principles underlying population phenomena are fundamentally identical in man and in other animals. The logistic curve rediscovered by Pearl and Reed in 1920 (originally discovered by Verhulst (1839) nearly a century before) as a result of a study of the growth of the human population of the United States has been shown to apply to a wide range of lower organisms.

The fact that there is an optimum population for animals which is neither the maximum nor the minimum must be as true of human beings as it is of the other animals. One is tempted to speculate as to the possible similarity between certain characteristics observable among human populations today and the abnormal behaviour apparently produced among certain of the lower animals by crowding. Pearl, who had given so much thought to this subject, was inclined to believe that there were evidences of discomfort associated with the present density of human population and asked, "Can it be honestly denied that on a world-wide view, *unrest* is the dominant characteristic of human behavior today?" That was in 1937. He hoped that mankind might be able to evolve techniques of living happily and contentedly at high population densities in a physically limited universe.

POPULATION STUDIES IN PROGRESS ON CANADIAN ANIMALS

Population studies in Canada to date have not been commensurate with either the need or the opportunity. In no country perhaps are fluctuations in animal populations more pronounced than in Canada. This is especially true in the case of fluctuations of a cyclic or periodic nature. These, of course, are characteristic of all northern areas but we are surely not going to be satisfied to wait for others to solve them for us These periodic fluctuations present a special challenge to us for two reasons. One is because they are especially characteristic of Canada at least as far as the New World is concerned and the other is because they offer such opportunities for prediction of population trends.

Although population studies have been entirely inadequate in Canada, some useful work has been done and a number of projects have been started which, if properly supported and expanded, promise to yield valuable results in the future.

Some of the researches of the Fisheries Research Board of Canada concerned with populations have already been cited. Some others are as follows:

The Pacific Biological Station has for many years carried out a comprehensive programme of sampling the commercial catch of herrings and pilchards in waters off the British Columbia coast with the object of determining population trends with particular reference to size and age-class changes. Previous to the last herring fishing season the Station was able, as the result of the analysis of data based on a study of extensive samples, to predict the entrance of a rich year-class into the fishery in one of the fishing areas. Racial studies supported by tagging experiments had proved that there is comparatively little intermingling of herring constituting the populations of such fishing areas as the west coast of Vancouver Island, the east coast of Vancouver Island, a central area, and a northern area. It is therefore necessary in following population changes to sample the stock in each area separately.

In contrast to this condition in the herring of a number of rather discrete populations existing, there is free interchange in the case of another Pacific coast fish, the pilchard, between Canadian and Californian fishing grounds.

Most of the pilchard spawning takes place rather less than one hundred miles off shore between San Diego and Point Conception in southern California and north and south migrations satisfactorily explain the supply of pilchards to Canadian waters. Each succeeding summer as the young pilchards grow older they move farther north along the California coast returning southward again toward autumn. The larger fish go farther north and they take a longer time to get back. The largest fish usually appear off the British Columbia coast in summer, off San Francisco in early winter, and still farther south in midwinter.

Sampling and age determinations have shown that the pilchard supply is recruited by annual broods of widely varying strengths. The broods of 1939, 1938, and 1937 were strong or fairly strong but were succeeded by weak ones, which is the chief reason for the poor pilchard catch during the past year or two.

At the Atlantic Biological Station of the Fisheries Research Board, lobsters have been tagged so as to be recognizable until they moult. By determining the proportion which such tagged lobsters constitute of later catches not only is an estimate of actual population in an area obtained but also the rate of mortality due to fishing. By this means it has been determined that in twelve areas studied, from forty to eighty per cent of the market-size lobsters are caught during the fishing season.

Creel censuses in eight lakes in Charlotte County, New Brunswick, carried out over a period of years have shown that, in spite of stocking, the yield to anglers was on the average less than one pound per acre. Similar studies, although for shorter periods, on the same type of lake in other parts of New Brunswick and Nova Scotia have revealed yields usually of the same order. In contrast, two ponds in a more fertile region, Prince Edward Island, yielded respectively forty and ninety-two pounds of trout per acre.

Estimates of the population of smelts (*Osmerus mordax*) supporting the fishery in the estuary of the Miramichi River, New Brunswick, have also been attempted through the tagging and release in 1946 of 50,000 individuals. Only 0.36 per cent of over 500,000 smelts taken in the commercial fishery during the next ten days were found to bear tags. Examination of samples of smelts constituting the spawning run to brooks entering the main river and the north-east branch failed to reveal a single tagged fish entering these sections. This is interpreted as indicating that the population in the estuary towards the close of the fishing season probably represents the advance run of spawning fish which go farthest up the river to tributaries entering the south west branch and that fish which spawn in tributaries of the main river and north-west branch enter the estuary after the close of fishing operations. These studies are only in their preliminary stages but they indicate the sort of information that may be derived from population studies of this kind.

The Ontario Fisheries Research Laboratory of the Department of Zoology, University of Toronto, in co-operation with the Ontario Department of Lands and Forests has carried out creel censuses on many of the lakes of Algonquin Park over a period of eleven years.

The character of the sockeye populations of several of the river systems of British Columbia has been recorded for thirty years. This study which was commenced by Dr. C. H. Gilbert in 1915 was continued for years by Dr. and Mrs. W. A. Clemens and more recently by Dr. Clemens (Clemens, 1945). Since for the solution of many population problems records over long periods are necessary, studies such as those on the sockeye salmon populations are especially valuable. It is to be hoped that records of this kind which have been in progress for so many years will not be allowed to lapse.

The Bureau of Animal Population, Oxford, with the co-operation of the Northwest Territories Administration, the National Parks Bureau of the Department of Mines and Natural Resources, and the Hudson's Bay Company is carrying out two questionnaire inquiries. One is entitled "Canadian Arctic Wild Life Enquiry." The results of this have been published annually in the *Journal of Animal Ecology* since 1938 (Chitty *et al.*, 1938-45). The results of the other, entitled "The Snowshoe Rabbit Enquiry," are being published in the *Canadian Field-Naturalist* (Elton *et al.*, 1933-43).

The Hudson's Bay Company, both independently and in co-operation with the Bureau of Animal Population at Oxford and the Royal Ontario Museum of Zoology, is contributing significantly to our knowledge of the populations of Canadian fur bearers. Since 1925 many of the Company's posts have furnished annual reports on fluctuations of wild life within their areas.

A questionnaire inquiry similar to that of the Bureau of Animal Population but with special reference to Ontario and including a wider range of birds and mammals has been carried out by the Royal Ontario Museum of Zoology since 1935. As Elton (1942) says: "The questionnaire system is still the only method which gives quickly and conveniently a general picture of fluctuations over vast areas."

A special feature of the Museum's questionnaire project since 1938 has been the accumulation of information on the periodic appearance of snowy owls in southern Canada and the northern United States. The Museum is headquarters of a co-operative programme for the compilation of data from Manitoba and Minnesota in the west to Washington, D.C., and Nova Scotia in the east (Snyder, 1943).

The Royal Ontario Museum of Zoology has also been carrying on annual censuses of small mammals in Algonquin Park since 1939.

Dr. W. Rowan of the University of Alberta has been actively interested in the problem of periodicities in hares, grouse, and other animals for many years and has accumulated a great deal of data on the subject, especially as it applies to Alberta.

RECOMMENDATIONS

The study of animal populations is of sufficient importance to Canada to warrant the establishment of an organization for the promotion of such studies. Such an organization should either be a research institution comparable with the Bureau of Animal Population at Oxford or a body charged with the responsibility of stimulating and co-ordinating work in this field by research institutions in various parts of the country. Present research agencies engaged in population studies need to be strengthened and much new work undertaken.

Extension and improvement of population studies should include:

1. Provision for better commercial fisheries and fur statistics. Regions for which statistics are separately recorded should be natural production units. When these have been established on a proper basis, records for them should be continuous and consistent.

2. The undertaking of studies designed to improve the accuracy of censusing various types of aquatic and terrestrial animals.

3. Plans for making and recording annual censuses over a long period of years of a number of important mammals, birds, fish, insects, and other animals.

4. Greatly increased provision for the study of the parasites and diseases of animals in nature since parasites and disease play such an important part in the fluctuations of animal numbers.

Few fields of research in Zoology offer better opportunities for making contributions of both practical and theoretical importance than does the field of animal populations and the factors affecting them.

REFERENCES

Allee, W. C. (1931): Animal aggregations. Chicago.

Allen, J. A. (1903): Report on the mammals collected in north-eastern Siberia by the Jessup North Pacific Expedition. Bull. Amer. Mus., **19**: 101.

Anderson, R. M. (1937): Mammals and birds of the western Arctic district, Northwest Territories, Canada. Canada's western northland, p. 110. Dept. Mines and Resources, Ottawa.

——— (1935): Mammals of the eastern Arctic and Hudson Bay. Canada's eastern Arctic, p. 88. Dept. Interior, Ottawa.

Babcock, J. P. (1902): Report of the Fisheries Commissioner for British Columbia for the year 1902.

Bailey, Vernon (1924): Breeding, feeding and other life habits of meadow mice (*Microtus*). J. Agric. Res., **28**: 523-35.

Banfield, A. W. F. (1947): A study of the winter feeding habits of the short-eared owl (*Asio flammeus*) in the Toronto region. Can. J. Res. **25D**: 45-65.

British Columbia (1944): Report of the Fisheries Department for 1944, p. M83.

Butler, L. (1942): Fur cycles and conservation. Trans. Seventh North Amer. Wildlife Conf., pp. 463-70.

Cameron, T. W. M. (1938): Animal parasites and wild animals. XIIIth Intern'l Vet. Congress: 13-22.

Chapman, R. N. (1928): The quantitative analysis of environmental factors. Ecology, **9**: 111.

——— (1931): Animal ecology. New York.

Chitty, D. (1938): Canadian Arctic wild life enquiry, 1936-37. J. Anim. Ecol., **7**: 381-94.

——— (1939): Canadian Arctic wild life enquiry, 1937-38. J. Anim. Ecol., **8**: 247-60.

——— (1940): Canadian Arctic wild life enquiry, 1938-39. J. Anim. Ecol., **9**: 227-42.

Chitty, D. and Chitty, H. (1941): Canadian Arctic wild life enquiry, 1939-40. J. Anim. Ecol., **10**: 184-203.

——— (1942): The snowshoe rabbit enquiry, 1939-40. Can. Field-Nat., **56**: 17-21.

Chitty, D. and Elton, C. (1937a): Canadian Arctic wild life enquiry, 1935-36. J. Anim. Ecol., **6**: 368-85.

——— (1937b): The snowshoe rabbit enquiry, 1935-36. Can. Field-Nat., **51**: 63-73.

——— (1938): The snowshoe rabbit enquiry, 1936-37. Can. Field-Nat., **52**: 63-72.

——— (1939): The snowshoe rabbit enquiry, 1937-38. Can. Field-Nat., **53**: 63-70.

——— (1940): The snowshoe rabbit enquiry, 1938-39. Can. Field-Nat., **54**: 117-24.

Chitty, D. and Nicholson, M. (1942): Canadian Arctic wild life enquiry, 1940-41. J. Anim. Ecol., **11**: 270-87.

——— (1943): The snowshoe rabbit enquiry, 1940-41. Can. Field-Nat., **57**: 64-8.

Chitty, H. (1943): Canadian Arctic wild life enquiry, 1941-42. J. Anim. Ecol., **12**: 163-72.

——— (1944): The snowshoe rabbit enquiry, 1941-42. Can. Field-Nat., **57**: 136-41.

Chitty, H. and Chitty, D. (1945): Canadian Arctic wild life enquiry, 1942-43 J. Anim. Ecol., **14**: 37-45.

Clarke, C. H. D. (1936): Fluctuations in numbers of ruffed grouse, *Bonasa umbellus* (Linne), with special reference to Ontario. Univ. Tor. Stud., Biol. Ser., no. 41.

Clemens, W. A. (1945): Contributions to the life-history of the sockeye salmon (No. 30). Rpt. B.C. Fish. Dept. for 1944, pp. M32-43.

Clements, F. E. and Shelford, V. E. (1939): Bio-ecology. New York.

Cowan, I. McT. (1938): The fur trade and the fur cycle, 1825-57. B.C. Hist. Quart., Jan., 1938.

Cox, W. T. (1936): Snowshoe rabbit migration, tick infestation and weather cycles. J Mamm., **17**: 216-21.

Criddle, N. (1930): Some natural factors governing the fluctuations of grouse in Manitoba. Can. Field-Nat., **44**: 77-80.

Cross, E. C. (1940): Periodic fluctuations in numbers of the red fox in Ontario. J. Mamm., **21**: 294-306.

Darwin, C. R. (1859): On the origin of species by means of natural selection. London.

Davis, D. E. (1937): A cycle in northern shrike emigrations. Auk, **54**: 43-9.

De Lury, Ralph (1930): Sunspots in relation to fluctuations in grasshoppers and grouse at Aweme, Manitoba. Can. Field-Nat., **44**: 120.

Dymond, J. R. (1944): Spread of the smelt (*Osmerus mordax*) in the Canadian waters of the Great Lakes. Can. Field-Nat., **58**: 12-14.

Elton, C. (1924): Periodic fluctuations in the numbers of animals: their causes and effects Brit J. Expt'l Biol., **2**: 119-63.

——— (1927): Animal ecology. London.

——— (1930): Animal ecology and evolution. Oxford.

——— (1933a): Matamek conference on biological cycles: abstract of papers and discussions. Matamek Factory, Canadian Labrador.

——— (1933b): The Canadian snowshoe rabbit enquiry, 1931 32. Can. Field-Nat., **47**: 64-9; 84 6.

——— (1934): The Canadian snowshoe rabbit enquiry, 1932-33. Can. Field-Nat., **48**: 73-8.

——— (1935a): Fluctuations in wild life. Canada's eastern Arctic, pp. 62-6. Dept. Interior, Ottawa.

——— (1935b): An epidemic among voles (*Microtus*) on the Scottish border in the spring of 1934. J. Anim. Ecol., **4**: 277-88.

——— (1942): Voles, mice and lemmings: problems in population dynamics. Oxford.

Elton, C. and Nicholson, M. (1942a): Fluctuations in numbers of the muskrat (*Ondatra zibethica*) in Canada. J. Anim. Ecol., **11**: 96-126.

——— (1942b): The ten-year cycle in the number of lynx in Canada. J. Anim. Ecol., **11**: 215-44.

Elton, C. and Swynnerton, G. (1935): The Canadian snowshoe rabbit enquiry, 1933-34. Can. Field-Nat., **49**: 79-85.

——— (1936): The Canadian snowshoe rabbit enquiry, 1934-35. Can. Field-Nat., **50**: 71-81.

Errington, Paul L. (1945): Some contributions of a fifteen-year local study of the northern bobwhite to a knowledge of population phenomena. Ecol. Monographs, **15**: 1-34.

——— (1946): Predation and vertebrate populations. Quart. Rev. Biol., **21**: 144-77; 221-45.

Foerster, R. E. (1929): An investigation of the life history and propagation of the sockeye salmon (*Oncorhynchus nerka*) at Cultus Lake, British Columbia. No. 1. Introduction and run of 1925. Contr. Can. Biol. Fish., **5**: 3-35.

——— (1938): An investigation of the relative efficiencies of natural and artificial propagation of sockeye salmon (*Oncorhynchus nerka*) at Cultus Lake, British Columbia. J. Fish. Res. Board of Canada, **4**: 151-61.

Foerster, R. E. and Ricker, W. E. (1941): The effect of reduction of predaceous fish on survival of young sockeye salmon at Cultus Lake. J. Fish. Res. Board of Canada, **5**: 315-36.

Gause, G. F. (1934): The struggle for existence. Baltimore.

Green, R. G., Larson, C. L., and Bell, J. F. (1939): Shock disease as the cause of the periodic decimation of the snowshoe hare. Amer. J. Hyg., **30B**: 83-102.

Green, R. G. and Shillinger, J. E. (1935): Progress report of wildlife disease studies for 1934. Trans. 21st Amer. Game Conf., pp. 397-401.

Griswold, F. G. (1929): The life-history of the Canadian salmon (*Salmo salar*). Norwood, Mass. Privately printed.

Gross, A. O. (1927): The snowy owl migration of 1926-27. Auk, **44**: 479-93.

——— (1931): Snowy owl migration 1930-31. Auk, **48**: 501-11.

Hall, E. R. (1927): An outbreak of house mice in Kern County, California. Univ. Calif. Pub. Zool., **30**: 189-203.

Hamilton, W. J. (1937): The biology of microtine cycles. J. Agric. Res., **54**: 779-90.

——— (1939): American mammals. New York.

Hart, J. L. (1933): Catch statistics of the British Columbia pilchard. Biol. Board of Canada, Bull. 38

——— (1943): The pilchard, *Sardinops caerulea* (Girard) on Canadian fishing grounds with special reference to an unusual abundance of young fish. Trans. Roy. Soc. Can., **37**, sec. V: 55-73.

Hewitt, C. G. (1921): The conservation of the wildlife of Canada. New York.

Hjort, J. (1914): Fluctuations in the great fisheries of northern Europe. Cons. Perm. Int. pour l'Exp. de la Mer, Rapp. et Proc.-verb., **20**: 1-228.

——— (1926): Fluctuations in the year-classes of important food fishes. J. Cons. Perm. Int. pour l'Exp. de la Mer, **1**: 5-38.

Howell, A. B. (1923): Periodic fluctuations in the numbers of small mammals. J. Mamm., **4**: 149-55.

Huntington, E. (1931): The Matamek conference on biological cycles. Science, **74**: 299-35.

——— (1932): Matamek conference on biological cycles. Matamek Factory, Canadian Labrador.

Huntsman, A. G. (1931): The maritime salmon of Canada. Biol. Board of Canada, Bull. 21.

——— (1937): The cause of periodic scarcity in Atlantic salmon. Trans. Roy. Soc. Can., **31**, sec. V: 17-28.

——— (1938): Relative strength of year-classes of certain fishes of the western Atlantic: North American Atlantic salmon. Cons. Perm. Int. pour l'Exp. de la Mer, Rapp. et Proc.-verb., **101**: 11-15.

——— (1941): Cyclical abundance and birds versus salmon. J. Fish. Res. Board of Canada, **5**: 227-35.

Huntsman, A. G. and Dymond, J. R. (1940): Pacific salmon not established in Atlantic waters. Science, **91**: 447-9.

Leopold, A. (1931): Game survey of the north central states. Madison, Wisc.

Lotka, J. A. (1920): Analytical note on certain rhythmic relations in organic systems. Proc. Nat. Acad. Sci., **6**: 410.

MacFarlane, R. (1905): Notes on mammals collected and observed in the northern Mackenzie River district, Northwest Territories of Canada. Proc. U.S. Nat. Mus., **28**: 673-764.

MacLulich, D. A. (1936): Sunspots and abundance of animals. J. Roy. Astron. Soc. Can., **30**: 233-46.

——— (1937): Fluctuations in the numbers of the varying hare (*Lepus americanus*). Univ. Tor. Stud., Biol. Ser., no. 43.

Mooney, G. S. (1861): Remarks on Frazer River fisheries. Edinburgh New Phil. J., **13**: 164-5.

Murie, A. (1934): The moose of Isle Royale. Univ. Mich. Mus. Zool., Misc. Pub. 25.

Nice, M. M. (1933): The theory of territorialism and its development. Fifty years' progress of American ornithology, 1883-1933, pp. 89-100. Lancaster, Pa.

Patterson, G. (1887): "The plague of mice" in Nova Scotia and P. E. Island. Can. Rec. Sci., **2**: 472-80.

Pearl, R. (1937): On biological principles affecting populations: human and other. Amer. Nat., **71**: 50-68.

Pearl, R. and Reed, L. J. (1920): On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proc. Nat. Acad. Sci., **6**: 275.

Phelps, E. B. and Belding, D. L. (1931): A statistical study of the records of salmon fishing on the Restigouche River. New York.

Poland, H. (1892): Fur bearing animals in nature and commerce. London.

Pritchard, A. L. (1937): Variation in the time of run, sex proportions, size and egg content of adult pink salmon (*Oncorhynchus gorbuscha*) at McClinton Creek, Masset Inlet, B.C. J. Biol. Board of Canada, **3**: 403-16.

——— (1938): Transplantation of pink salmon (*Oncorhynchus gorbuscha*) into Masset Inlet, B.C. in lean years. J. Fish. Res. Board of Canada, **4**: 141-50.

Rae, J. (1890): Notes on some of the birds and mammals of the Hudson's Bay Co.'s territory and of the Arctic coast of America. J. Linn. Soc. (Zool.), **20**: 136-45.

Ross, R. (1911): The prevention of malaria. London.

Russell, F. S. (1930): The seasonal abundance and distribution of the pelagic young of teleostean fishes caught in the ring-trawl in offshore waters in the Plymouth area. J. Mar. Biol. Assoc., **16**: 707-22.

Seton, E. T. (1911): The Arctic prairies. Toronto.

——— (1928): Lives of game animals, vol. IV. New York.

Severtzoff, S. A. (1934): On the dynamics of populations of vertebrates. Quart. Rev. Biol., **9**: 409-37.

Shaler, E. A. and Krefting, L. W. (1946): The present status of moose on Isle Royale. Trans. Eleventh North Amer. Wildlife Conf., pp. 296-308.

Snyder, L. L. (1935): A study of the sharp-tailed grouse. Univ. Tor. Stud., Biol. Ser., no. 40.

——— (1943): The snowy owl migration of 1941-1942. Wilson Bull., **55**: 8-10.

Speirs, J. M. (1939): Fluctuations in numbers of birds in the Toronto region. Auk, **56**: 411-19.

Stevenson, J. C. (1944): The smelt situation in the upper Great Lakes, Ontario, May, 1943. Can. Field-Nat., **58**: 128-9.

Uvarov, B. P. (1921): A revision of the genus *Locusta* L. (*Pachytylus* Fieb.) with a new theory as to the periodicity and migrations of locusts. Bull. Ent. Res., **12**: 135-63.

——— (1928): Locusts and grasshoppers. Imperial Bureau of Entomology, London.

VERHULST, P. F. (1839): Notice sur la loi que la population suit dans son accroissement. Corr. math. et phys. publ. par A. Quiltelet, **10**: 113.

VOLTERRA, V. (1926): Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. Mem. Accad. Naz. Lincei (Sci. Fis. Mat. e Nat.), Ser. 6, **2**(3): 31-112.

WAYSON, N. E. (1927): An epizootic among meadow mice in California, caused by the bacillus of mouse septicemia or of swine erysipelas. Pub. U.S. Health Repts., **42**: 148-93.

WELLS, A. Q. (1937): Tuberculosis in wild voles. Lancet, May 22: 1221.

WHELAN, R. V. (1939): Vole plague at Smoky Falls, Ontario. Can. Field-Nat., **53**: 53-5.

WHITE, H. C. (1939): Bird control to increase the Margaree River salmon. Fish. Res. Board of Canada, Bull. 58.

WING, L. W. (1935): Wildlife cycles in relation to the sun. Trans. 21st Amer. Game Conf., pp. 345-63.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLI : SERIES III : MAY, 1947
SECTION FIVE

Effect on Growth and Basal Metabolic Rate of Excessive Amounts of Some of the B Vitamins in the Presence of Added Desiccated Thyroid

By JOHN ALLARDYCE, EWALD GORANSON, and EDWARD SINGER
Presented by A. H. HUTCHINSON, F.R.S.C.

DURING the past thirty years many workers have investigated various substances for their beneficial effects in thyroid therapy. A summary of this work prior to 1935 has been made by Cameron (1935). In 1939 Scrutinio reported that vitamin B_1 had a lowering effect on the basal metabolic rate in a hyperthyroid condition, a finding not in agreement with that of Frazier and Ravdin in 1938. In the last ten years an increasing amount of attention has been paid to the antithyrogenic action of the B vitamins with particular reference to their countering influence on weight lost, anorexia, and glycolysis (Cowgill and Palmieri, 1933; Drill, 1938; Drill and Hays, 1940; Drill and Overman, 1942; Drill, Overman, and Shaffer, 1942; Himwick, Goldfarb, and Cowgill, 1931; Sure and Buchanan, 1937). In 1941 Starr and Pomerenze investigated the possibility of an inhibitory hormone mechanism acting to reduce hyperthyroidism. They observed an occasional case where such a mechanism might be functioning but attempts to produce the inhibitory substance by the administration of desiccated thyroid, thyrotropic hormone, testosterone proprionate, and vitamins A and C met with no success. In 1943, Astwood, Sullivan, Bissell, and Tyslowitz and MacKenzie and MacKenzie showed that certain sulfonamides and thiourea-like drugs induced enlargement of the thyroid and lowering of the basal metabolic rates of animals. Later Griesbach and Purves (1943), Astwood and Bissell (1944), Franklin, Lerner, and Chaikoff (1944) and Rawson, Tannheimer, and Peacock (1944) showed that these drugs interfered with the metabolism of iodine and that the thyroids of the animals receiving them were unable to take up iodine or synthesize thyroxine. The present investigation over the past four years was undertaken to ascertain the effects on growth and basal metabolic rates of Wistar albino rats of giving excessive amounts of p-aminobenzoic acid, calcium pantothenate, pyridoxine, nicotinic acid amide, riboflavin, or thiamine hydrochloride in the presence of added desiccated thyroid.

METHODS

The Wistar albino rats used in this investigation were housed in wire cages in a room thermostatically controlled between 25° and 26° C. To further avoid any seasonal effect, the light in the rat room was electrically controlled to give the same length of illumination at the same time each day. Water was supplied *ad libitum*. In the first part of the investigation (Figs. 1 and 2), each rat was given 18 gm. "Purina Fox Chow Checkers" per day. In the second part of the investigation (Figs. 3 and 4), the diet was reduced to 13 gm. per day. In the balance of the investigation (Figs. 5, 6, and 7), Fox Chow was supplied *ad libitum*. Hyperthyroidism was induced by daily doses of desiccated thyroid (1/10 grain unless otherwise stated) thoroughly incorporated with the Fox Chow. When vitamin supplements were added (1 mg. per day) these also were incorporated with the Fox Chow. The apparatus used in the determination of basal metabolic rates was constructed after the plan of Schwabe and Griffith (1938) with certain modifications (Allardyce, Aldous, Cooper, Pratt, and Sutherland, 1942). This apparatus was set up in a small room thermostatically held at 29° C. The rats were held in this room for at least an hour before being placed in the metabolism chamber. With the exception of the results shown in Fig. 3, all are for male rats. All rats were fasted twenty to twenty-two hours before their basal metabolic rates were determined. Calculations of the B.M.R. were made using Rubner's formula for determining the surface area of the rat, Benedict's assumption (1930) that the R.Q. of a rat that has been fasting twenty-four hours is close to 72, and Lusk's caloric value for a litre of oxygen at standard temperature and pressure as 4.702 calories (1924).

RESULTS

Preliminary survey. As there was some choice about the length of fast to which the rats should be subjected, the temperature at which the B.M.R. should be determined, the age of the rats to be used, and the time of day at which the tests should be carried out, some preliminary work was done to aid making a decision on these points.

In keeping with the results of Kingdon, Bunnell, and Griffith (1942) it was found that the B.M.R. curve did not level out during twenty-four hours of fasting. In consequence it was considered best to make all determinations as nearly as possible after the same number of hours of fasting. The lowest values were obtained after twenty hours of fasting and in consequence this time was selected as giving the most nearly basal values.

Although Benedict and MacLeod (1929) regarded 28° C. as the temperature of "thermal neutrality" for the rat while Swift and Forbes (1939) reported 30° C. as the "critical temperature," it was found that it would be very difficult to distinguish B M.R.'s at 28° and 30° C. Hence in this work the intermediate temperature, 29° C., has been used.

Davis (1937) has reported that the oxygen consumption per kilogram of rat fell off rapidly during the first four months and more slowly thereafter. In the present survey, B.M.R. determinations were made on a group of rats six weeks old, weekly thereafter until the twelfth week,

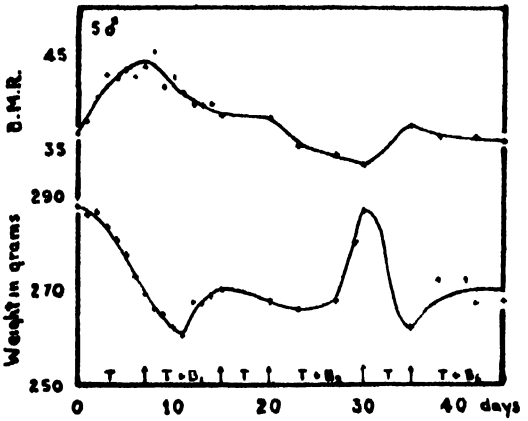


FIGURE 1.- Showing the changes in B M.R and weight when 1/10 grain desiccated thyroid was given daily and vitamins B_1, B_2, and B_6 as indicated.

and finally at twenty-eight weeks. From this it was seen that there was a rapid drop in B.M.R. between the sixth and tenth weeks. Thereafter the drop by comparison was much smaller.

Horst, Mendel, and Benedict (1934) found that the B.M.R. of the rat is subject to a diurnal variation, being higher in the early morning and late afternoon and low at midday. They recommended that B.M.R. measurements be confined to the period from 10 A.M. to 4 P.M. Agreement with this was obtained and determinations confined to this interval.

Antithyrogenic action of thiamine hydrochloride, riboflavin, and pyridoxine. From Fig. 1 it would seem that daily administration of thiamine hydrochloride has an immediate countering action to the elevating effect of desiccated thyroid on the B.M.R. On withdrawing the thiamine hydrochloride supplement but continuing with the desiccated thyroid, no further change showed in the B.M.R. during the next five days. When a supplement of riboflavin was added, the B.M.R again dropped, going even below the original level. Withdrawal of the riboflavin supplement permitted the B.M.R. to return to its original level. Then on adding a supplement of pyridoxine a small drop in B.M.R. occurred. The effect of added thiamine hydrochloride on weight loss while not immediate caused the rats after four days' treatment to start regaining their lost weight. Withdrawal of the thiamine hydrochloride supplement permitted the lowering effect of desiccated thyroid on weight to appear again. Adding riboflavin caused, after some delay, a sharp rise in weight to the original level. Subsequent withdrawal of the riboflavin supplement again sent the weight down sharply, as in the first part of the experiment. Finally addition of pyridoxine caused the rats to start regaining lost weight at once but the curve tended to flatten out and the gain after ten days' treatment was very similar to that seen with thiamine hydrochloride treatment. Of the three vitamins used in this experiment, riboflavin appeared to have the greatest antithyrogenic action.

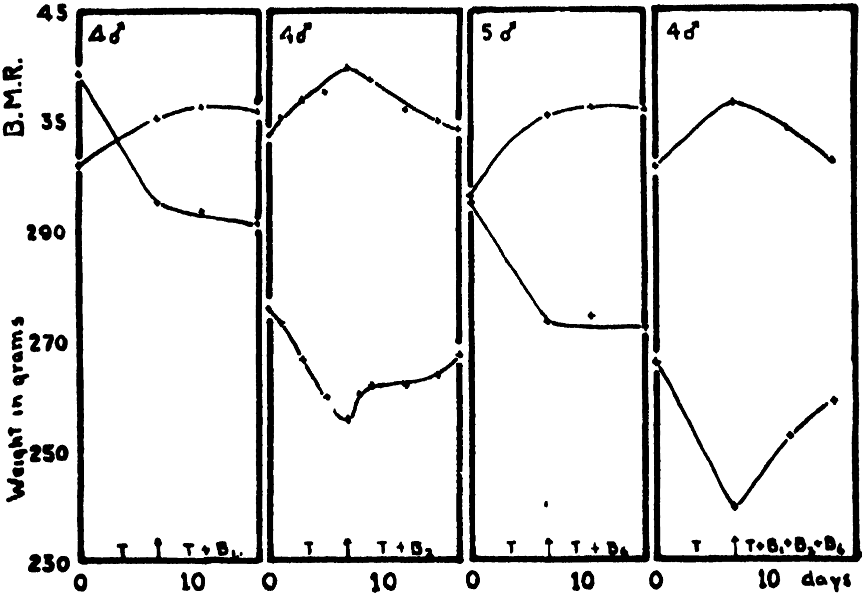
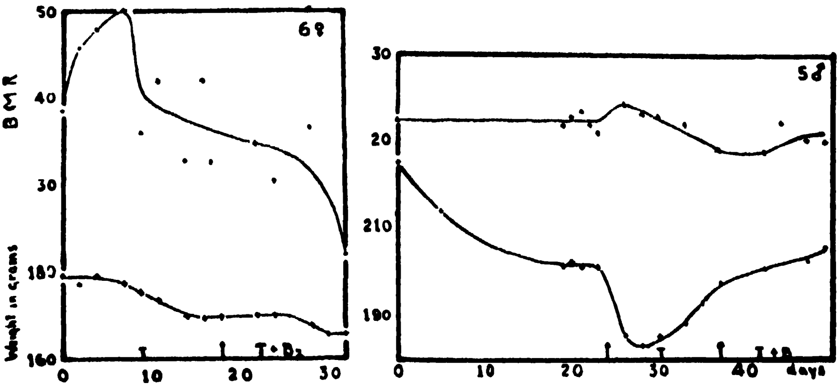


FIGURE 2. Showing the changes in B.M.R. and weight when 1/4 grain desiccated thyroid was given daily and vitamins B_1, B_2, and B_6 as indicated.

In Fig. 2 are shown the results of a shorter series of experiments using the same three vitamins. However, instead of switching from one vitamin supplement to another for the same rats, separate groups of rats were used. Further, the amount of desiccated thyroid used was increased to 1/4 grain daily. Again it was noted that riboflavin had greater antithyrogenic effect than thiamine hydrochloride or pyridoxine. A combined supplement of thiamine hydrochloride, riboflavin, and pyridoxine appeared no more effective than the use of riboflavin alone.

Difference in response between male and female rats. In comparing the response between female and male rats as shown in Figs. 3 and 4 respectively, it will be noted that the females exhibited a greater elevation of the B.M.R. on treatment with thyroid, were more erratic in the subsidence of the B.M.R., and showed a greater depression of the B.M.R. on treatment with riboflavin. With both the males and the females it will be noted that the B.M.R. subsided to a level even below the original values before the riboflavin supplement had been added and despite the continued daily addition to the diet of desiccated thyroid. In comparing the weight changes of these two groups of rats, it should be kept in mind that the rats were on a reduced diet (13 gm. per rat instead of 18 gm. as was the case in Figs. 1 and 2). This may account for the loss in weight shown by the males in Fig. 4 even before the addition of thyroid. It will be noted that the males suffered the greater loss in weight on addition of thyroid and that they recovered most of this loss before the riboflavin supplement was added; the females also showed some tendency to stop losing weight before the riboflavin supplement was added.



FIGURES 3 and 4—Showing the difference in response by female and male rats to 1/10 grain desiccated thyroid given daily and B_2 as indicated.

Difference in response between older and younger rats. In view of the results given in Figs. 3 and 4, which showed that rats appeared to be able to overcome the effect of daily addition of desiccated thyroid before the addition of the riboflavin supplement, four groups of male rats were set up. To the first group, which served as controls, Fox Chow alone was fed. The second group received Fox Chow plus desiccated

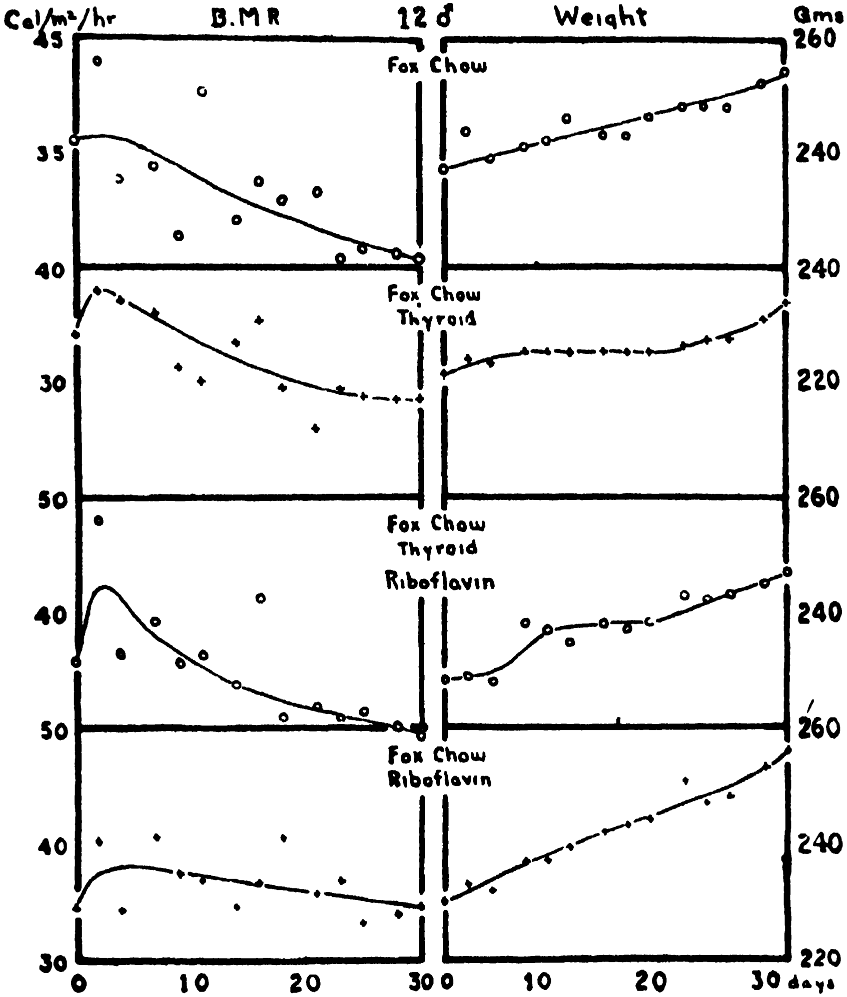


FIGURE 5.—Showing the ability of the rat to overcome the action of daily supplements of 1/10 grain desiccated thyroid.

thyroid. The third group received Fox Chow, desiccated thyroid, and riboflavin. The fourth group received Fox Chow and riboflavin. To ensure complete consumption of the thyroid and riboflavin, these were supplied mixed wtih only 2 gm. Fox Chow and when this had been eaten Fox Chow was added for consumption *ad libitum*. The results are shown in Fig. 5 with each point representing the average value for the rats in that group.

The greatest elevation of the B.M.R. came in the group receiving both thyroid and riboflavin, the second greatest elevation in the group receiving thyroid and the third greatest elevation in the group receiving riboflavin.

While the points for B.M.R. are somewhat erratic, there seems to be little doubt that over the thirty-day period of the test the B.M.R. tended to drop, the greatest drop appearing in the control group, the next greatest drop in the group receiving thyroid and riboflavin, and the third greatest drop in the group receiving thyroid. In the group receiving riboflavin, the B.M.R. tended to drop after the elevation at the start but unlike the other three curves this one, at the end of the thirty-day period, did not go below the original value.

Looking next at the weight changes, it is seen that the control group gained steadily showing an average gain of 17 gm. per rat over the thirty-day period. The group receiving thyroid stopped gaining weight for eleven days in the middle of the test period and then began to gain weight once more, finishing with an average gain of 13 gm. The group receiving thyroid plus riboflavin also tended to stop gaining weight in the middle of the test period but this tendency was not so marked as in the thyroid group and it went on to gain in the last part of the test period to finish with an average 18.5 gm. gain. The group receiving riboflavin showed a steady gain and finished with an average gain of 26 gm.

To ascertain how younger rats would respond to addition of desiccated thyroid and how supplements of thiamine hydrochloride and of riboflavin would affect this response, four groups of male rats were set up in which the average weight per rat was 77.5 gm. All groups received Fox Chow *ad libitum* throughout the thirty-seven days of the test. During the first eight days no thyroid or vitamin supplements were given to any group. The first group, which served as controls, was continued on the same diet throughout the full test period but the other three groups received the following daily supplements: the second group received thyroid; the third group, thyroid plus thiamine hydrochloride; the fourth group, thyroid plus riboflavin. From the ninth to the twenty-third day the desiccated thyroid supplement was 1/10 grain and from the twenty-third to the thirty-seventh day it was 1/2 grain. Vitamin supplements (thiamine hydrochloride and riboflavin) were given from the ninth day on. The results are shown in Fig. 6. The values shown for the B.M.R. on the ninth day are the averages for each group over the first eight days. Each point on a curve is the average value for the rats in that group at that particular time.

It will be noted that the B.M.R. of the control group remained practically constant throughout the test. In the second group, which received the thyroid supplement, the B.M.R. rose slightly during the first week that thyroid was given and then flattened out in the second week. At the end of this second week, when the thyroid supplement was increased to 1/2 grain daily, the B.M.R. rose more sharply than in the first week and then began once more to level off. In the third group, which received thyroid and thiamine hydrochloride supplements, the response in the first week was similar to that in the group receiving thyroid only, while in the second, third, and fourth weeks respectively the levelling off, the second rise, and the second levelling off were less. In the fourth group, which received thyroid and riboflavin supplements, there was no levelling off in the second week but a further rise, while the increase in the thyroid supplement at the end of the second week caused no further rise but resulted in a tendency for the B.M.R. to level off in the third and fourth weeks.

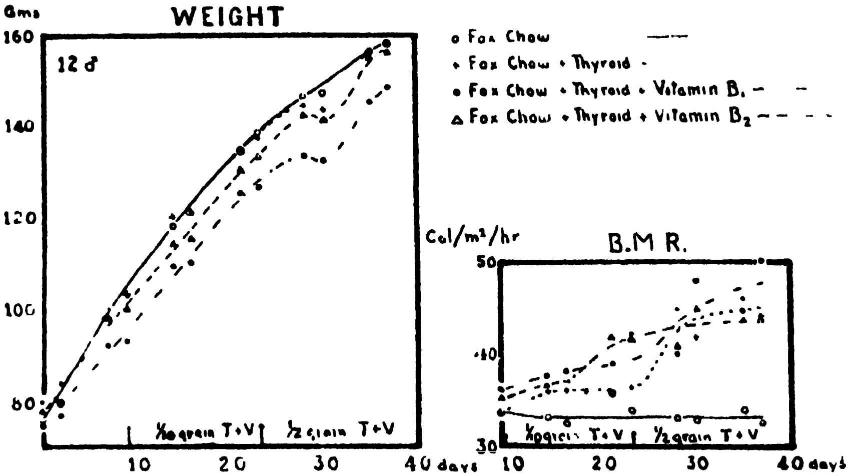


FIGURE 6 -Showing the lower response of younger rats to daily supplements of 1/10 grain desiccated thyroid and the effect of increasing the thyroid dosage to 1/2 grain

In reference to weight changes it will be seen that the addition of 1/10 grain thyroid and of vitamin supplements resulted in curves that,

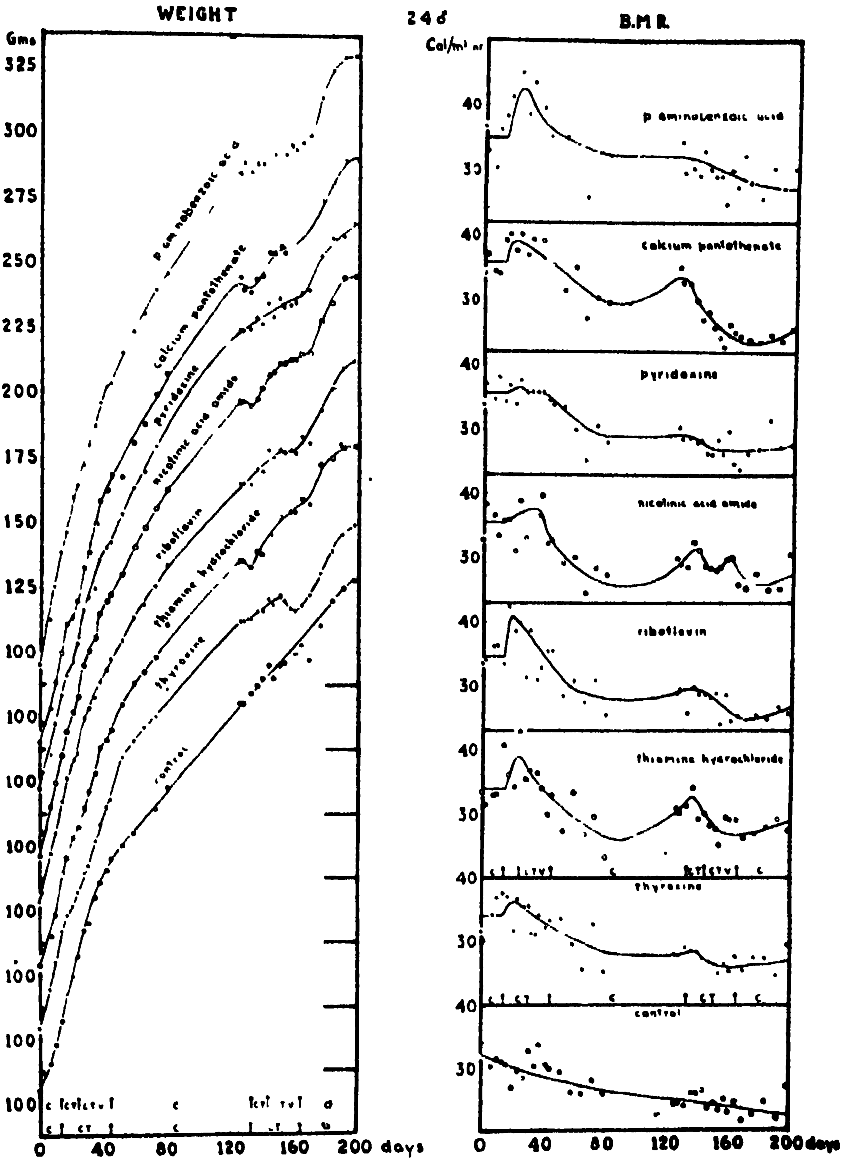


FIGURE 7.—Showing the effect of 1/10 gram desiccated thyroid supplements daily and of the inclusion of different vitamins in an initial and a repeated series. Controls were given Fox Chow only. The group labelled thyroxine got Fox Chow (*C*) and Fox Chow plus thyroid (*C T*) as indicated. The other six groups got *C*, *C T*, and *C T V* (Fox Chow, thyroid, and vitamin named) as indicated.

for all practical purposes, were similar for each group. When the thyroid supplement was increased to 1/2 grain there followed shortly a depression in each curve which, however, was soon overcome and the curves continued their original general direction.

Comparative effects of p-aminobenzoic acid, calcium pantothenate, pyridoxine, nicotinic acid amide, riboflavin, and thiamine hydrochloride. This series of tests had two purposes: first, to check the results already obtained with thyroxine, thiamine hydrochloride, riboflavin, and pyridoxine; and second, to extend the comparison to p-aminobenzoic acid, calcium pantothenate, and nicotinic acid amide. For the tests twenty-four male rats, each weighing approximately 100 gm., were selected and the tests were carried out over a 198-day period. The rats were divided into eight groups. Group 1 was fed Fox Chow only throughout the 198 days. Group 2 received in addition 1/10 grain desiccated thyroid per rat daily from the fourteenth to the forty-fifth day and again from the 133rd to the 165th day. The other six groups received the same as Group 2 and in addition 1 mg. per rat daily of the B vitamin indicated in Fig. 7 from the twenty-fourth to the forty-fifth day and again from the 144th to the 165th day. Each point on each curve in Fig. 7 represents the average value for the rats in that group at that time.

To save space in plotting the weight changes, the curves for these changes have been closely grouped, with each successive one, starting around the 100 gm. level, placed twenty-five points above the previous one. Looking at the curve for the control group first, it will be seen that the greatest rate of gain in weight came in the first forty days of the test, at the end of which time the rats approached the 200 gm. level. Thereafter the gain in weight continued but at a less rapid rate, just passing the 300 gm. level by the end of the test period. By comparing this curve with each of the other seven, several interesting points stand out. This comparison was made easier by superimposing the control curve on each of the other seven and in turn the thyroid curve on each of the curves obtained with thyroid plus a vitamin supplement; however, space hardly permits this here.

When the weight curve for the group getting thyroid supplements only was compared with the control curve, it was found that the former ran parallel to and just above the control until the twenty-fourth day (ten days after introducing the thyroid supplement), when it crossed below the control to again parallel it until the thyroid supplement was withdrawn. The thyroid curve then crossed back above the control and ran parallel to it until the thyroid supplement was again introduced. This caused the thyroid curve to cross below the control curve a second time, dropping below the latter further than it did the first time. On both occasions the thyroid curve tended to resume its original direction even before the thyroid supplement was withdrawn, and the withdrawal appeared to aid this recovery still further.

Turning next to the weight curve for the group getting both thyroid and thiamine hydrochloride supplements, it was seen that the pattern followed, up to the point where the thyroid supplement was introduced for the second time, was very similar to that just described above when thyroid supplement alone was given. At this point the decrease in rate of gain in weight, while more definite than when the supplements were first given, came sooner and was less than in the group receiving thyroid supplement only.

Consideration of the group receiving both thyroid and riboflavin supplements revealed little if any indication of a change in rate of gain in weight the first time these supplements were added. At a later stage, when these supplements were again added, there was a definite decrease in rate of gain but this was less marked than when thyroid alone had been given.

There is little to differentiate the weight curves for the other four groups in the first half of the test, but in the second half they tend to follow either one or the other of the two patterns described above where thiamine hydrochloride or riboflavin supplements were added.

At the end of the 198 days the control rats averaged 303 gm.; those receiving desiccated thyroid supplements alone weighed 299 gm.; the rats receiving thyroid plus vitamin supplements all weighed more than the controls, with those receiving p-aminobenzoic acid being heaviest at 329 gm. and those getting thiamine hydrochloride the lightest at 309 gm.

Turning next to the basal metabolic rate curves it will be seen that there is a general tendency for the B.M.R. of the control group to drop gradually over the 198-day period. Because of this and of the somewhat erratic nature of the B.M.R., a starting-point in each group was obtained by taking the average value for each group over a fourteen-day period (five determinations on each rat) prior to the giving of any supplements. Unlike the response seen in the weight curves, the greater changes in B.M.R. came in the first half of the experiment rather than in the second half. This is clearly shown where thyroid supplement alone was given. It will also be seen that the B.M.R., after elevation, tends to come down to and even go below its original level despite the continued daily addition of thyroid supplements, and finally, after removal of the thyroid supplements, the B.M.R. curve tends to flatten out or rise to the level shown prior to the addition of the thyroid supplement.

Inasmuch as all rats receiving thyroid supplements were given the same amount, any differences in the height of the curves obtained with the first supplements of thyroid are not significant and are likely due to individually divergent degrees of sensitivity to thyroid. However, it will be seen that, with the possible exception of those rats getting thyroid plus pyridoxine supplements, the B.M.R. of rats given thyroid plus B-vitamin supplements was slower in returning to the original level than where thyroid supplements alone were given. This is particularly noticeable where thyroid and nicotinic acid amide supplements were given. In this case, in the first half of the experiment the peak in the curve was delayed over a week, and in the second half an additional peak was obtained. It should also be noted that the rats receiving thyroid plus p-aminobenzoic acid supplements showed a greater sensitivity to thyroid in the first half of the experiment than did any of the other groups, while in the second half the same group failed to show any elevation of the B.M.R. when the supplements were repeated.

DISCUSSION

Consideration of the results shown in Figs. 1 and 2 suggested that thiamine hydrochloride, riboflavin, and pyridoxine supplements were effective in reducing the action of desiccated thyroid supplements on the elevation of B.M.R. and on the loss of weight and that, of the three vitamins used, riboflavin was the most effective. When this was investigated further it was found (Figs. 3 and 4) that, by giving the desiccated thyroid supplements over a longer period before adding the riboflavin supplements, the B.M.R. for both male and female rats dropped back and even below the original level without the riboflavin supplements and despite the continued administration of the thyroid, and further that the weight losses during the same period decreased or were corrected. Apparently the rats were able to adjust themselves fairly readily to the action of the added thyroid. This may be the explanation of the variation in results obtained by some investigators of the antithyrogenic properties of certain vitamins and other compounds.

Although both sexes of rats were able successfully to counter the action of added thyroid (Figs. 3 and 4), the elevation of B.M.R. was greater in females and the weight losses were greater in males. Response to thyroid supplement was also affected by age. While the rats were still growing rapidly, weighing under 150 gm., the addition of 1/10 grain desiccated thyroid daily or of thyroid plus thiamine hydrochloride or riboflavin had very little if any modifying influence on the rate of gain in weight (Fig. 6). Increasing the thyroid dosage to 1/2 grain produced a definite but brief depression in the growth curve. When the rats were older, weighing over 200 gm., and the growth curve was less steep, the slowing of the rate of gain in weight by the addition of 1/10 grain desiccated thyroid daily was quite definite and this was partially countered by the addition of riboflavin (Fig. 5). At the same time, the elevation of B.M.R. was greater in older rats than in younger ones. However, when a second series of 1/10 grain desiccated thyroid supplements daily was given to the same rats after an interval of eighty-eight days (Fig. 7), the elevation of B.M.R. was not greater, as expected on the basis of age, but in all cases was less. This would lend support to the earlier suggestion that the rats were able to develop some tolerance to the thyroid given.

Although the rats were able, without the aid of vitamin supplements, to overcome the action of daily doses of desiccated thyroid, nevertheless inclusion of riboflavin with the thyroid supplement caused the rats to increase in weight more rapidly than when it was not included (Fig. 5). Riboflavin was more effective than thiamine hydrochloride or pyridoxine in aiding recovery of weight losses (Figs. 1 and 2). Riboflavin was also more effective than the other two in lowering the B.M.R. (Fig. 2). In Fig. 7, where the comparison was extended to include nicotinic acid amide, calcium pantothenate, and p-aminobenzoic acid, it was evident that, although the recovery from the action of thyroid supplements began before the vitamin supplements were added, the inclusion of the latter facilitated the recovery since they caused greater gains in weight and greater lowering of the elevated B.M.R. than occurred where the thyroid alone was added. Attempts, on the basis of the data obtained in Fig. 7, to assess the relative merits of the vitamins used in countering thyroid supplements were based on four points: (*a*) how soon the weight curves of the test groups, after the first vitamin supplements were given, began to exceed that for the control group; (*b*) how far the elevated B.M.R. dropped by the time the first vitamin supplements were withdrawn; (*c*) how far altogether the elevated B.M.R. dropped with the first series of vitamin supplements; and (*d*) how far the elevated B.M.R. dropped by the time the second series of vitamin supplements were withdrawn. This placed riboflavin as the most effective vitamin and it was followed in turn by thiamine hydrochloride, p-aminobenzoic acid, nicotinic acid amide, calcium pantothenate, and pyridoxine.

SUMMARY

The effect on growth and basal metabolic rate in the rat of excessive amounts of riboflavin, thiamine hydrochloride, p-aminobenzoic acid,

nicotinic acid amide, calcium pantothenate, and pyridoxine in the presence of added desiccated thyroid has been investigated.

The response was affected by the age of the rat, its sex, and whether or not it had been subjected to similar treatment before. Younger rats responded less than did older rats. Females exhibited greater changes in B.M.R. while males showed greater changes in weight. When the series of thyroid supplements was repeated after an interval, the effect on the elevation of B.M.R. was less than in the first series.

The rats readily developed some tolerance to the action of daily doses of desiccated thyroid and this was facilitated by inclusion with the thyroid of supplements of any one of the vitamins named above. Of these vitamins riboflavin was the most effective and the others followed in the order named.

REFERENCES

Allardyce, J., Aldous, J., Cooper, W., Pratt, J., and Sutherland, E. (1942). Amer. J. Physiol., **137**: 761.

Asdwood, E. B. and Bissell, A. (1944). Endocrinology, **34**: 282.

Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R. (1943). Endocrinology, **32**: 210.

Benedict, F. G. (1930). J. Nutrition, **3** 161.

Benedict, F. G. and MacLeod, G. (1929). J. Nutrition, **1**: 343.

Cameron, A T. (1935). Recent advances in endocrinology. Philadelphia.

Cowgill, G. R. and Palmieri, M. L. (1933). Amer J. Physiol., **105**: 146.

Davis, J. E. (1937). Amer. J. Physiol., **119**: 28.

Drill, V. A. and Hays, H. W (1940). Proc. Soc. Expt'l Biol. and Med., **43**: 450.
Drill, V. A. and Hays, H W. (1940). Proc. Soc. Expt'l Biol. and Med., **43**: 450.
Drill, V. A. and Overman, R. (1942). Amer. J. Physiol., **135**: 474.

Drill, V. A., Overman, R., and Shaffer, C. B. (1942). Endocrinology, **31**: 245.

Franklin, A. L., Lerner, S. R. and Chaikoff, I. L. (1944). Endocrinology, **34**: 265.

Frazier, W. D. and Ravdin, I. S. (1938). Surgery, **4**: 680.

Griesbach, W. E. and Purves, H. D. (1943). Brit. J. Expt'l Pathol., **24**. 174.

Himwick, H. E., Goldfarb, W., and Cowgill, G. R. (1931). Proc. Soc. Expt'l Biol. and Med., **28** · 646.

Horst, K., Mendel, L. B., and Benedict, F. G. (1934). J. Nutrition, **7**: 277.

Kingdon, C., Bunnell, I. L. and Griffith, F. R. (1942). Amer. J. Physiol., **137**: 114.

Lusk, G. J. (1924). J. Biol. Chem., **59**:41.

MacKenzie, C. G. and MacKenzie, J. B. (1943). Endocrinology, **32**: 185.

Rawson, R. W., Tannheimer, J. F., and Peacock, W. (1944). Endocrinology, **34**: 245.

Schwabe, E. L. and Griffith, F. R. (1938). J. Nutrition, **15**: 187.

Scrutinio, L. (1939). Bol. Soc. Ital. di Biol. sper., **14**: 563.

Starr, P. and Pomerenze, H. (1941). Ann. Intern'l Med., **15**: 226.

Sure, B. and Buchanan, K. S. (1937). J. Nutrition, **13**: 513.

Swift, R. W. and Forbes, R. M. (1939). J. Nutrition, **18**: 307.

MÉMOIRES DE LA SOCIÉTÉ ROYALE DU CANADA
TOME XLI : TROISIÈME SÉRIE : MAI 1947
SECTION CINQ

Utilisation digestive et teneur en matières cellulosiques de la ration

Par ANDRÉ GASNIER
Présenté par LOUIS BERGER, M.S.R.C.

TOUS les auteurs sont d'accord pour admettre que plus le régime alimentaire est riche en matières cellulosiques (crude fibre), plus la digestibilité de l'ensemble de la ration et de chacun des composants de celle-ci (protides, lipides, glucides) est faible. Ce sont, notamment, les innombrables études réalisées sur les animaux de la ferme, en vue de déterminer la valeur nutritive des aliments du bétail, qui ont permis d'arriver à cette conclusion; et, pour ne citer qu'un exemple, Axelsson (1942), analysant les résultats de près de huit cents études de digestibilité chez le bœuf, le mouton et la chèvre, montre que, pour chaque augmentation de 1% des matières cellulosiques de la ration, l'utilisation digestive de la matière organique totale diminue de 0.81%, celle des protéines de 0.45% et celle des extractifs non azotés de 1.08%.

Les études de nutrition que nous avons entreprises sur le lapin montrent que cette espèce animale ne fait pas exception à la règle générale; elles indiquent en outre que la loi de diminution de la digestibilité en fonction de la teneur en matières cellulosiques du régime n'est pas unique pour toute la gamme des concentrations que l'on peut imposer sans danger à l'animal.

Ce sont ces résultats qui font l'objet du présent mémoire.

Utilisation digestive du lapin en fonction de la teneur du régime en matières cellulosiques

Toutes nos mesures ont été réalisées sur des animaux parfaitement adaptés, d'une part, au régime que nous voulions étudier, d'autre part, aux cages à métabolisme utilisées pour de telles recherches (Gasnier, 1939). Nous avons déterminé l'utilisation digestive de la matière sèche totale de la ration en calculant le rapport

$$\text{C.U.D.} = \frac{\text{Matière sèche ingérée—Fecès sèches}}{\text{matière sèche ingérée}} \times 100$$

pour des périodes d'au moins sept jours consécutifs de durée. Les régimes utilisés, qu'il serait trop long de décrire un à un, étaient tous composés d'aliments naturels du lapin, tels que: farine de luzerne, son, son mélassé, avoine, orge, paille, ou encore d'aliments composés du commerce enrichis de produits riches en matières cellulosiques comme, par exemple, la paille de blé ou la bourre de trèfle.

Notons enfin que la teneur en matières cellulosiques des différents régimes imposés aux animaux a été déterminée par la méthode de Weende et que les valeurs que l'on trouvera dans le tableau suivant représentent les moyennes de l'utilisation digestive mesurée chez au moins trois animaux adultes.

TENEUR EN MATIÈRES CELLULOSIQUES POUR 100 G. SECS DE RÉGIME ET UTILISATION DIGESTIVE DU LAPIN

Teneur en matières cellulosiques % g. secs	C.U.D. %	Teneur en matières cellulosiques % g. secs	C.U.D. %
7.1	75.7	17.6	54.4
8.9	70.6	18.1	47.9
10.7	68.0	18.7	53.2
12.6	65.0	21.0	46.0
14.7	63.9	27.6	43.7
15.7	60.4	28.6	43.2
16.1	58.2	38.3	41.7

Ainsi, chez le lapin comme chez les ruminants, le fait d'augmenter la teneur de la ration en matières cellulosiques diminue considérablement l'utilisation digestive.

Lois de variation de l'utilisation digestive en fonction de la teneur du régime en matières cellulosiques

Vient-on à placer sur un graphique (Fig. 1) les points figuratifs des mesures groupées dans le tableau précédent, on s'aperçoit que, jusque vers une teneur en matières cellulosiques de l'ordre de 20 g. pour 100 g. secs, les points s'alignent très convenablement, d'une façon continue. Pour les valeurs supérieures à 20 g. pour 100 g. secs, les points s'alignent encore mais sur une droite totalement différente de la première. En calculant les équations de ces deux droites par la méthode d'ajustement des moindres carrés, on trouve que:

(1) Pour les rations à teneur en matières cellulosiques inférieure à 20 g. pour 100 g. secs, la digestibilité diminue de 2.1% pour toute augmentation de 1% de cette teneur;

(2) Pour les rations à teneur en matières cellulosiques supérieure

à 20 g. pour 100 g. secs, la digestibilité ne diminue, dans les mêmes conditions, que de 0.2%.

D'autre part, le calcul montre que les deux droites représentatives de ce phénomène de diminution de la digestibilité se coupent en un point correspondant à une concentration de 22.2 g. de matières cellulosiques pour 100 g. secs de ration.

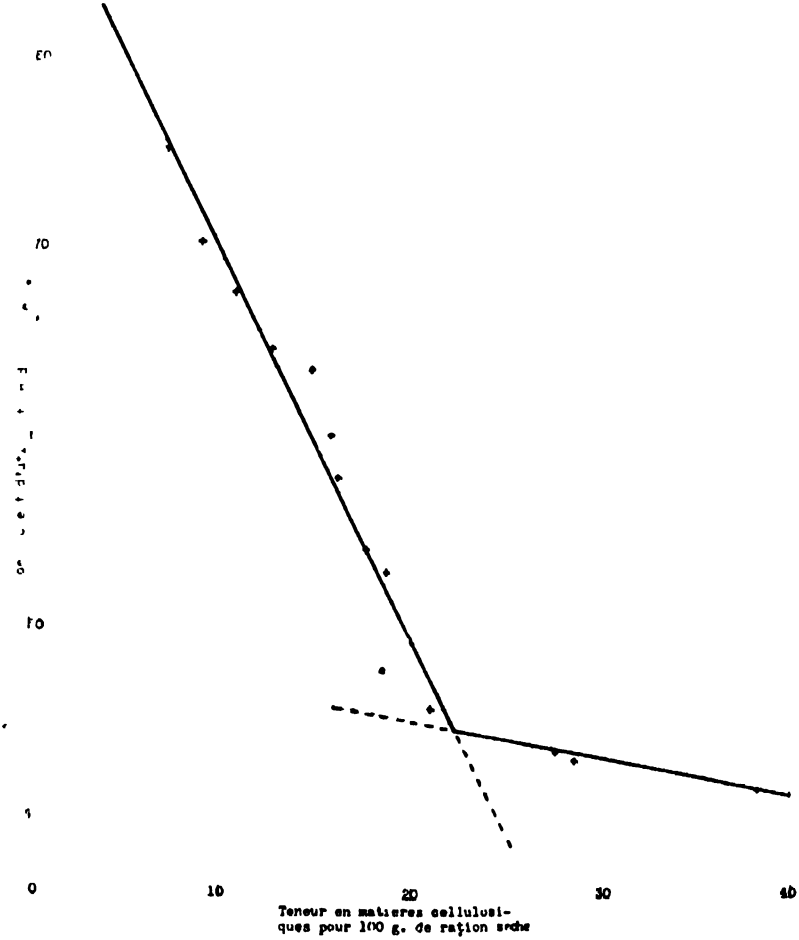


FIGURE 1.—Variation de l'utilisation digestive chez le lapin en fonction de la teneur du régime en matières cellulosiques.

Discussion

(1) Etant donné le nombre relativement faible de mesures que nous avons pu réaliser, il nous a paru utile de rechercher dans la

littérature si des résultats analogues aux nôtres ont déjà été signalés. A vrai dire, les renseignements sur l'utilisation digestive par le lapin des aliments mis habituellement à la disposition de cette espèce sont peu nombreux. C'est le travail de Le Roy Voris et de ses collaborateurs (1940) qui nous semble le plus important au point de vue qui nous occupe.

Les tables que ces auteurs publient dans leur mémoire et qui donnent la composition et la digestibilité de quarante-sept aliments du lapin nous ont permis de constater que la diminution de la digestibilité en fonction de la teneur en matières cellulosiques de la ration suit encore ici deux lois linéaires différentes, suivant que cette teneur est inférieure ou supérieure à 20 g. pour 100 g. de ration sèche. Les diminutions de la digestibilité sont respectivement, de 1.8 et 0.9% pour chaque augmentation de 1% de la teneur en matières cellulosiques de la ration sèche et les deux droites d'ajustement que l'on peut tracer se coupent en un point correspondant à une teneur de 22.5% g. secs.

Les résultats de Le Roy Voris et de ses collaborateurs confirment donc ceux que nous avons précédemment rapportés; les différences que l'on peut noter entre les valeurs des pentes des deux droites représentatives du phénomène tiennent, selon nous, aux deux méthodes expérimentales utilisées: les auteurs américains déterminent, en effet, l'utilisation digestive d'un aliment donné alors que nous avons mesuré la valeur alimentaire d'une ration complète.

(2) Il est intéressant de savoir si, pour d'autres espèces animales, on retrouve ce même phénomène. Une revue sommaire de quelques tables de composition et de digestibilité des aliments du bétail (A.-M. Le Roy, 1944; *Food and Life*, 1939) nous a permis de montrer qu'il en est bien ainsi. Par exemple, pour les bovins, jusqu'à une teneur en matières cellulosiques de 25 g. % g. secs, la digestibilité de la matière organique diminue de 1% pour chaque augmentation de 1% de cette teneur en matières cellulosiques. Au-delà de la concentration «critique» de 25%, on n'enregistre plus, dans les mêmes conditions, qu'une diminution de 0.6% de la digestibilité.[1] Pour les ovins, les résultats sont très sensiblement comparables: 1% et 0.5%, la zône de changement brusque de la digestibilité se plaçant également aux environs de 25 g. de matières cellulosiques pour 100 g. de ration sèche.

---

[1] On peut noter que si l'on ne tient pas compte de l'existence de cette teneur «critique» et que l'on calcule la diminution de l'utilisation digestive sur l'ensemble des valeurs des tables, on trouve que, pour chaque augmentation de 1% de la teneur en matières cellulosiques de la ration, de 0 à 50%, la digestibilité de la matière organique diminue de 0.84%, résultat en parfait accord avec celui d'Axelsson que nous avons signalé plus haut.

Chez les porcins, comme chez les volailles, cette zône semble se situer vers 18% g. secs; on sait bien que ces espèces animales sont de très mauvais utilisateurs des aliments peu digestibles. Pour les volailles, en outre, la diminution de la digestibilité est très marquée—2.2%—lorsque la teneur en matières cellulosiques augmente de 1% depuis 0 jusqu'à 18 g. pour 100 g. de matières sèches, tandis qu'elle n'est plus que de 0.3% environ lorsqu'on dépasse la concentration «critique» de 18%.

(3) Il semble donc bien établi que, quelle que soit l'espèce animale, on retrouve le phénomène que nous avons mis en évidence chez le lapin. Ce fait est d'autant plus remarquable que l'on sait combien sont difficiles les mesures de digestibilité chez certaines espèces, combien il est hasardeux de compter en bloc ce qu'on est convenu d'appeler «matières cellulosiques» (crude fibre), groupant ainsi artificiellement, à cause de l'insuffisance des techniques, des composants de valeur digestive aussi différente que la lignine, les hémicelluloses et la cellulose proprement dite (Crampton et Maynard, 1938). Des recherches plus poussées dans ce domaine devraient, à coup sûr, permettre d'obtenir des résultats plus cohérents, des valeurs de digestibilité moins dispersées, et de déterminer avec plus d'exactitude ces concentrations «critiques» pour lesquelles la loi de l'utilisation digestive change brusquement de paramètres.

(4) Que la digestibilité totale d'une ration diminue au fur et à mesure qu'augmente la teneur en composants peu digestibles du régime, c'est là un phénomène auquel on doit logiquement s'attendre. Plus difficile à interpréter est le fait que, pour une augmentation donnée de cette teneur, disons pour une augmentation de 10%, on trouve, chez certaines espèces, une diminution de la digestibilité totale supérieure à 10%. Il semble qu'on puisse expliquer cette constatation en admettant que, à cause de l'encombrement plus grand du tractus digestif par des aliments de faible digestibilité, la vitesse du bol alimentaire se trouve augmentée: les aliments facilement attaquables par les sucs digestifs transitent trop vite pour pouvoir être utilisés et absorbés complètement.

C'est ainsi qu'Axelsson signale, chez les ruminants, que pour chaque augmentation de 1% de la teneur en matières cellulosiques de la ration, la digestibilité des extractifs non azotés diminue de plus de 1%, celle des lipides et des protides, respectivement de 0.8 et 0.45%, tandis que la digestibilité de la «crude fibre» ne diminue que de 0.15%.

Notre hypothèse paraît être confirmée par la différence considérable que nous avons relevée entre les ruminants, d'une part, et les monogastriques, d'autre part. Chez les premiers, outre que la flore du tractus digestif est spécialement adaptée à la digestion des aliments «encombrants», le volume de ce tractus est tel qu'une augmentation de la teneur de la ration en matières cellulosiques ne peut pas accroître considérablement la vitesse de transit du bol alimentaire. Il en est évidemment tout autrement pour les monogastriques chez lesquels le rapport du volume de l'estomac au volume de l'intestin est beaucoup plus petit que chez les polygastriques.

On peut en outre penser qu'une partie des sécrétions digestives se trouve retenue par les aliments de faible digestibilité et que, par suite, la quantité de ces sécrétions disponible pour les composants de la ration facilement attaquables va constamment en diminuant lorsqu'augmente la teneur du régime en matières cellulosiques.

Par conséquent, pour certaines espèces animales, la plus grande diminution de la digestibilité consécutive à une surcharge de la ration en aliments encombrants semble pouvoir s'expliquer, en partie au moins, par l'accélération du transit alimentaire.

(5) Mais, dans ces conditions, pourquoi enregistre-t-on, à partir d'une concentration donnée en matières cellulosiques, une brusque variation de la digestibilité, variation telle qu'une nouvelle augmentation de la teneur de la ration en aliments encombrants n'entraîne plus qu'une faible diminution de la digestibilité totale?

Pour répondre à cette question, on peut imaginer qu'à partir d'une certaine réplétion du tube digestif, la vitesse de transit du bol alimentaire ne peut plus augmenter, à moins qu'apparaissent des troubles de la digestion. Pour une telle vitesse du transit, l'attaque des aliments par les sécrétions gastrique et intestinales se réalise dans des conditions sensiblement identiques, quelle que soit la concentration en matières cellulosiques, entraînant l'utilisation et l'absorption d'une fraction à peu près constante de l'aliment offert à l'animal.

Ce raisonnement suppose que la composition des «matières cellulosiques» reste la même lorsqu'augmente, dans la ration, la teneur de ces composants; on sait bien qu'il n'en est pas nécessairement ainsi et que notamment, la teneur en lignine, pratiquement indigestible, peut atteindre des valeurs élevées dans certains aliments à haute teneur en matières cellulosiques. Rien d'étonnant alors à ce que, malgré une vitesse sensiblement constante du transit alimentaire, on enregistre encore une diminution de la digestibilité totale. Et l'hypothèse d'une inutilisation partielle des sécrétions digestives, à cause de la présence, dans le bol alimentaire, d'une quantité importante d'éléments faiblement digestibles, contribue également à expliquer la légère diminution de la digestibilité totale lorsque le régime renferme une grande proportion de matières cellulosiques.

Conclusions

On conçoit donc qu'il est non seulement inutile mais encore peu «économique» d'imposer aux animaux des régimes à haute teneur en composants de faible digestibilité, d'autant moins économique que l'espèce animale dont on se sert est moins apte à utiliser les «matières cellulosiques»--ce qui est le cas des monogastriques.

L'homme n'échappe pas à cette règle et dans la question si débattue du pain complet et du pain blanc, il ne faut pas perdre de vue que si un blutage très faible de la farine permet de fabriquer un pain à haute teneur en vitamines du groupe B, cette amélioration est compensée par un apport élevé de matières cellulosiques faiblement digestibles. Il en résulte que l'utilisation digestive de toute la ration, et notamment celle des lipides et des protides, se trouve nettement diminuée; de sorte que les besoins primordiaux en énergie et en protéines risquent de ne pas être couverts. La solution idéale paraît donc être, pour l'homme, de disposer d'un pain à teneur peu élevée en matières cellulosiques, suffisante toutefois pour assurer un péristaltisme normal, quitte à enrichir artificiellement la farine en vitamines. Il semble qu'un blutage de l'ordre de 75% remplisse ces conditions, assurant, même aux gros mangeurs de pain, une utilisation convenable de la ration sans fatigue anormale du tractus digestif.

Résumé

L'utilisation digestive de la ration dépend de la teneur du régime en matières cellulosiques (crude fibre). Lorsque cette teneur augmente progressivement, on enregistre d'abord une diminution importante de la digestibilité puis, à partir d'une certaine concentration, une diminution peu marquée du coefficient d'utilisation digestive. Un tel phénomène est particulièrement net chez les monogastriques, mauvais utilisateurs des matières cellulosiques.

On a proposé quelques hypothèses pour expliquer cette variation de la digestibilité et on a attiré l'attention sur le fait que, pour toutes les espèces, y compris l'homme, il n'est pas «économique» de chercher à employer des aliments à teneur élevée en matières cellulosiques.

RÉFÉRENCES BIBLIOGRAPHIQUES

Axelsson, J. (1942). Das Futterverdauungsvermögen der Wiederkäuer. Biedermann's Zentralbl., B—Tierernährung, **14**: 212-32.

Crampton, E. W. et Maynard, L. A. (1938). The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutrition, **15**: 383-95.

GASNIER, A. (1939). La nutrition du lapin domestique: rythme de l'ingestion. Rendement de la nutrition—Bull. Soc. Scient. Hygiène Alim., **27**: 85-110.

LE ROY, A.-M. (1944). Alimentation rationnelle des animaux domestiques. Agenda agricole 1944, pp. 14-31. Paris.

——— Cours professé à l'Institut National Agronomique, Paris. MSS.

UNITED STATES DEPARTMENT OF AGRICULTURE (1939). Food and life. Yearbook of agriculture 1939, pp. 839-43, 1067-76. Washington.

VORIS, LE ROY, MARCY, L. F., THACKER, E. J. et WAINIO, W. W. (1940). Digestible nutrients of feeding stuffs for the domestic rabbit. J. Agric. Res., **61**: 673-83.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLI : SERIES III : MAY, 1947
SECTION FIVE

Lethal Temperature Relations of Certain Fish of the Toronto Region[1]

By J. S HART

Presented by J. R DYMOND, F R S.C

THE quantitative study of the lethal effect of temperature on organisms may be conveniently divided into three main phases. The first is the establishment in the laboratory of a method of assessment which will permit prediction of the lethal effects of temperature in the field. The second is the application of this method to the description of species. The third is the determination of the relationship of this laboratory description to the distribution and the success of the species in nature.

The problem of laboratory assessment appears to be solved with the exception of the measurement of the rate of acclimation. The methods for studying lethal temperature relationships in the past have been, briefly, determination of (1) the temperature reached at death when heated at a constant rate, (2) the average survival time at a given constant temperature, and (3) the temperature which can be withstood for a given length of time. Reconciliation of these three methods was made by Fry, Hart, and Walker (1946). Their plan of description of the lethal effects of temperature is briefly as follows.

First of all they consider it necessary to determine the thermal zone in which the animal can live free from the lethal effects of temperature. This is the *zone of tolerance*. It is bounded by upper and lower *incipient lethal levels* where temperature is just beginning to have a lethal effect. The incipient lethal temperature rises as the acclimation temperature rises, but in the case of upper lethal temperatures, a point is reached where further rise in acclimation temperature fails to raise the incipient lethal temperature. The latter temperature is termed the *ultimate upper incipient level*, since it is the highest level to which the animal can be acclimated. At temperatures beyond the incipient lethal level the animal can live for a certain finite period of time; this has been termed the *resistance time*. The importance of the resistance time data is that these enable calculation of the death point in any changing thermal pattern beyond the incipient lethal level.

---

[1] This work was supported by a grant from the University of Toronto Research Fund to Professor F. E. J. Fry and carried out under his direction

The work reported here is a contribution to the second phase of the problem, that is, to the accumulation of comparative data on the lethal effects of temperature for a series of species. The final part of the problem, which concerns the significance of the differences found in relation to the survival of the species, will be the subject of future work.

METHOD AND MATERIAL

The experiments were carried out from November to April, 1945-6, on eight species of fish collected in the Toronto region. *Catostomus commersonnii*, *Rhinichthys atratulus*, *Semotilus atromaculatus*, *Pimephales promelas*, and *Notropis cornutus* were obtained from the Don River at Thornhill, Ontario. *Hyborhynchus notatus*, *Notropis atherinoides* and the perch, *Perca flavescens*, were caught in the Etobicoke Creek near Lake Ontario, in the Chippewa Creek (Welland River) at Welland, Ontario, and in the Black Creek at Lake Simcoe, respectively. All the fish were stored in hatchery troughs for various periods of time prior to use. The weight and age of the fish killed are summarized in Table V.

The method employed was the same as that described by Fry *et al.* (1946) for *Salvelinus fontinalis*, which in turn was developed from methods used by many previous workers. Essentially, it consisted in subjecting samples of fish to high or low temperatures in the lethal and sub-lethal range after they had been maintained at constant temperatures for periods ranging from four to twenty days. Certain species became acclimated very slowly and tests at many points were repeated before equilibrium was reached.[2] Five fish per sample were used except in the case of *Rhinichthys* acclimated to 5°, 10°, and 15° C., where ten fish per sample were used. The acclimation temperatures were 5°, 10°, 15°, 20°, and 25° C. for all the species except *Pimephales* and *Perca*. In *Pimephales* the temperatures used were 10°, 20°, 30° C., and in *Perca* 5°, 15°, and 25° C.

Following acclimation to a given temperature, a sample of five fish was placed directly into another thermostat maintained at the desired temperature. Running water and compressed air were used in all tanks. Glass-covered electric heaters maintained the temperatures to within 0.1° C.

The experiments in the lethal temperature tanks were extended until all the individuals had died, or, if 100 per cent mortality was not expected, until sufficient time had elapsed for death to have occurred as judged by extrapolation from the results at other temperatures. However, no experiments were carried on longer than about 8,000 minutes in upper lethal tests owing to uncertainties as to the cause of death, since the fish were occasionally attacked by fungus in such long-term tests. Tests at lower lethal temperatures were usually carried out for twenty-four hours unless the fish showed signs of dying after that period. In such cases the experiments were continued for longer periods.

---

[2] Subsequent evidence suggests that in certain cases acclimation had not been complete at the upper levels even in the time allowed.

The time of death of each individual was recorded and the median (50 per cent) mortality time was determined by calculating mean log mortality or by inspection after plotting the time to death of each fish against the per cent mortality in probits. It was indicated previously (Fry *et al.*, 1946) that for the purpose the geometric mean mortality is equivalent to the 50 per cent mortality.

No correlation was found between order of death and weight or age, even in the common sucker where some trend appeared to be exhibited. In all other cases there was no evidence of any trend at all. The data for the sucker are displayed in Table I. It is interesting to note that there is a strong trend in the average size, but no significant trend in the average weight composition by classes. This appears to be due to the overriding effect of a few large fish.

TABLE I

Weight classes of *Catostomus commersonnii* in 3 gm intervals in relation to order of death. The sample shown below includes approximately 70 per cent of the total number of suckers killed

Order of death	*Weight Class* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	*Mean weight gm.*
1	3	1		5	4	1		2	1		1	1			13.80
2	4		4	2	3	1		1			1	2		1	14.54
3	1	3	5	1	4	2	1		1	1					11.44
4	4	2	5	3	2		1		1						11.37
5	3	1	5	4	4	1	1								9.06

For rows 1 and 5: $\chi^2 = 18.74$ $n = 15$ $P = .2$ or $.3$

For rows 1 and 2, and 4 and 5: $\chi^2 = 16.0$ $n = 15$ $P = .3$

The incipient lethal temperatures were estimated by the method of dosage mortality, the special modification of de Beer (1945) being used. This is a graphical technique in which the mortality in probits is plotted against the logarithm of the temperature and both the median and standard error are determined by the use of special weighting scales constructed from the original tables given by Bliss.

RESULTS

1. *Upper and lower incipient lethal temperatures*

The upper and lower incipient lethal temperatures for the eight species are shown in Table II. Similar data are also included for *Carassius* (Fry *et al.*, 1942), *Salvelinus* (Fry *et al.*, 1946), ***Ameiurus*** (Brett, 1944), and *Girella* (Doudoroff, 1942), species previously studied. The data for the present species are displayed in Figs. 1 and 2 in the manner first used by Fry *et al.* for *Carassius*. This method of illustrating the data by plotting the upper and lower lethal temperatures against acclimation temperature presents an excellent picture of the thermal range for any species. Of the species studied so far, there are several characteristics common to all which were pointed out originally for *Carassius*. These points are:

(*a*) A rising incipient upper lethal temperature with rising acclimation temperatures up to a certain level. The relation has always been found linear to date.

(*b*) Above a certain acclimation temperature there is no further increase in the incipient upper lethal temperature. This has been termed the ultimate upper lethal temperature, and it is also the ultimate temperature to which the species can be acclimated.

(*c*) A rising incipient lower lethal temperature with rising acclimation temperature until the latter reaches the ultimate incipient level. This relation between lower lethal and acclimation temperature is also linear and the slope is steeper than that of the upper lethal temperatures.

(*d*) The lower lethal level is below the freezing point of fresh water. Hence determination of the ultimate incipient lower lethal level, in the species studied so far, has not been carried out.

Whereas these characteristics are common to all the species described here, there are several characters which can be seen to differ quantitatively from species to species. These are:

(*a*) The ultimate incipient upper lethal temperatures. These ranged from 29.3° C. for *Catostomus* and ***Rhinichthys*** to 33.3° C. for ***Hyborhynchus***. These fish are thus intermediate between ***Salvelinus*** (25.3°C.) on the one hand and *Carassius* (41°C.) on the other.

(*b*) The length of the "flat" portion of the upper lethal curve. The range over which there is change in the incipient upper lethal with changing acclimation temperatures is much restricted in certain

TABLE II

Incipient lethal temperatures and thermal tolerance. Upper (U) and lower (L) incipient lethal temperatures are given for each acclimation temperature. Thermal tolerance is given as the area contained by the diagrams of Figs. 1 and 2. Species marked * taken from the literature. Minutes to reach the incipient upper lethal are given in brackets. The error figures represent 2 × standard deviation.

Species		*Upper and Lower Incipient Lethal Temperatures*						*Thermal tolerance*
		Acclimation Temperatures						
		5	10	15	20	25	30	
**Salvelinus fontinalis*	U	23.7	24.1	25.0	25.3	25.3		625
	L					0.5		
**Girella nigricans*	U		28.7 (12°C.)		31.4		31.4 (28°C.)	800
	L		5.0		8.0		13.0	
**Carassius auratus*	U	29.0	30.8	32.8	34.8	36.6	38.6	1220
Catostomus commersonnii	U	26.3 ± .5 (310)	27.7 (310)	29.3 ± .3 (310)	29.3 ± .1 (2000)	29.3 (8000)		770
	L				2.5 ± .4	6.0 ± .6		
Rhinichthys atratulus	U	26.5 ± .9 (900)	28.8 ± .3 (1100)	29.6 ± .3 (3000)	29.3 (9500)	29.3 (20000)		790
	L				2.2 ± .5	5.0		
Semotilus atromaculatus	U	24.7 (250)	27.3 ± .4 (400)	29.3 ± .1 (600)	30.3 (1000)	30.3 ± .1 (1900)		808
	L				0.7	4.5		
Hyborhynchus notatus	U	26.0 ± .3 (350)	28.3 (650)	30.6 (1200)	31.7 (2000)	33.3 ± .5 (9000)		884
	L			1.0	4.2	7.5		
Pimephales promelas	U		28.2 (1300)		31.7 ± .3 (1800)		33.2 ± .3 (9000)	903
	L				1.5		10.5	
Notropis cornutus	U	26.7 ± .5 (300)	28.6 ± .4 (600)	30.3 ± .3 (1100)	31.0 (2500)	31.0 ± .4 (5500)		803
	L			0.0	3.7	7.8		
Notropis atherinoides	U	23.2 ± .4 (400)	26.7 ± .6 (450)	28.9 ± .4 (500)	30.7 ± .3 (2500)	30.7 ± .4 (10000)		747
	L			1.6 ± .4	5.2 ± .7	8.0 ± .6		
**Ameiurus nebulosus*	U	28.6	30.2	31.8	33.1	35.0	36.5	1162
	L				− 1.0	1.3	3.7	
Perca flavescens	U	21.3 ± .3 (190)	25.0 ± .2 (210) (11°C.)	27.7 ± .5 (230)		29.7 (5000)		742
	L					3.7		

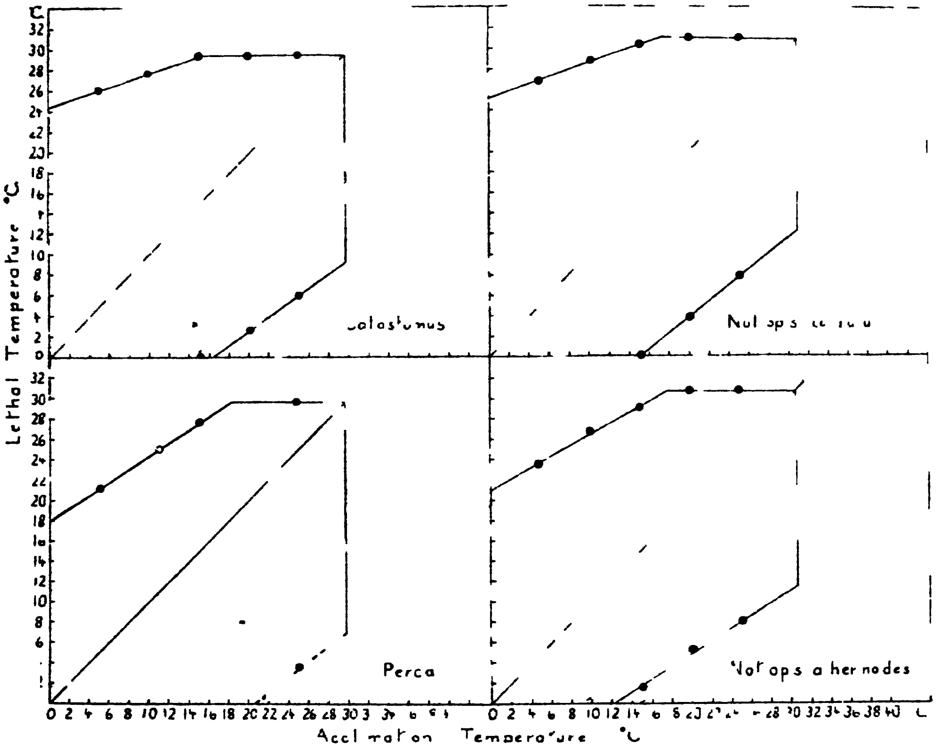


FIGURE 1.—The relation between acclimation temperature and the upper and lower incipient lethal temperatures for *Catostomus commersonnii*, *Perca flavescens*, *Notropis cornutus*, and *Notropis atherinoides*. Dotted lines indicate approximate range for 10 per cent and 90 per cent mortality.

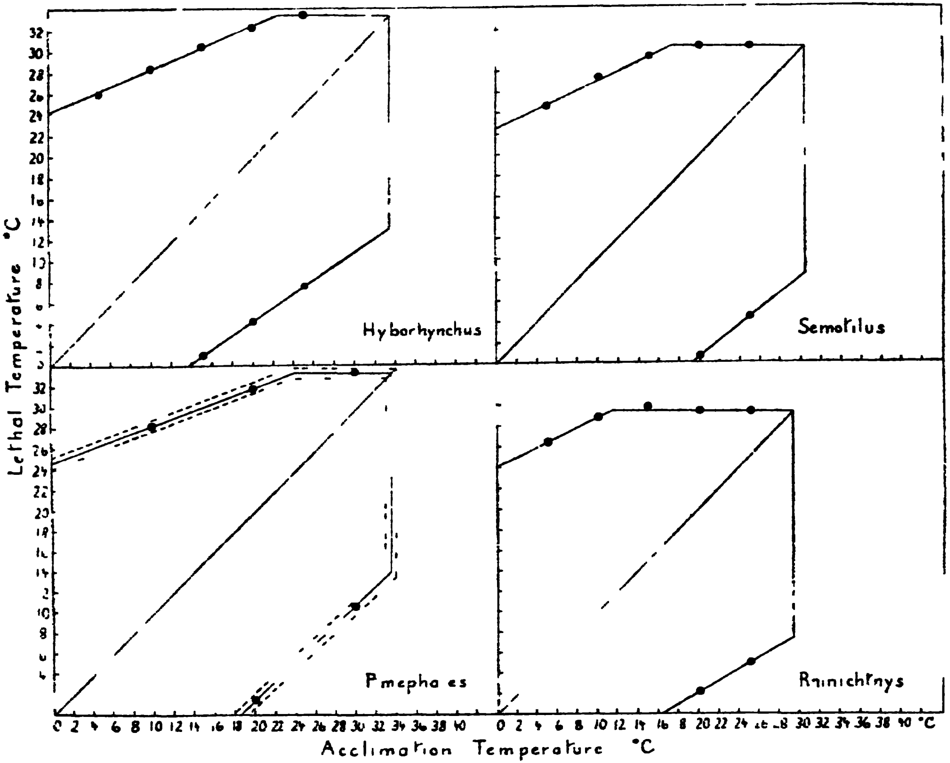


FIGURE 2.—The relation between acclimation temperature and upper and lower incipient lethal temperatures for *Hyborhynchus*, *Semotilus*, *Pimephales*, and *Rhinichthys*. Dotted lines indicate approximate range for 10 per cent and 90 per cent mortality.

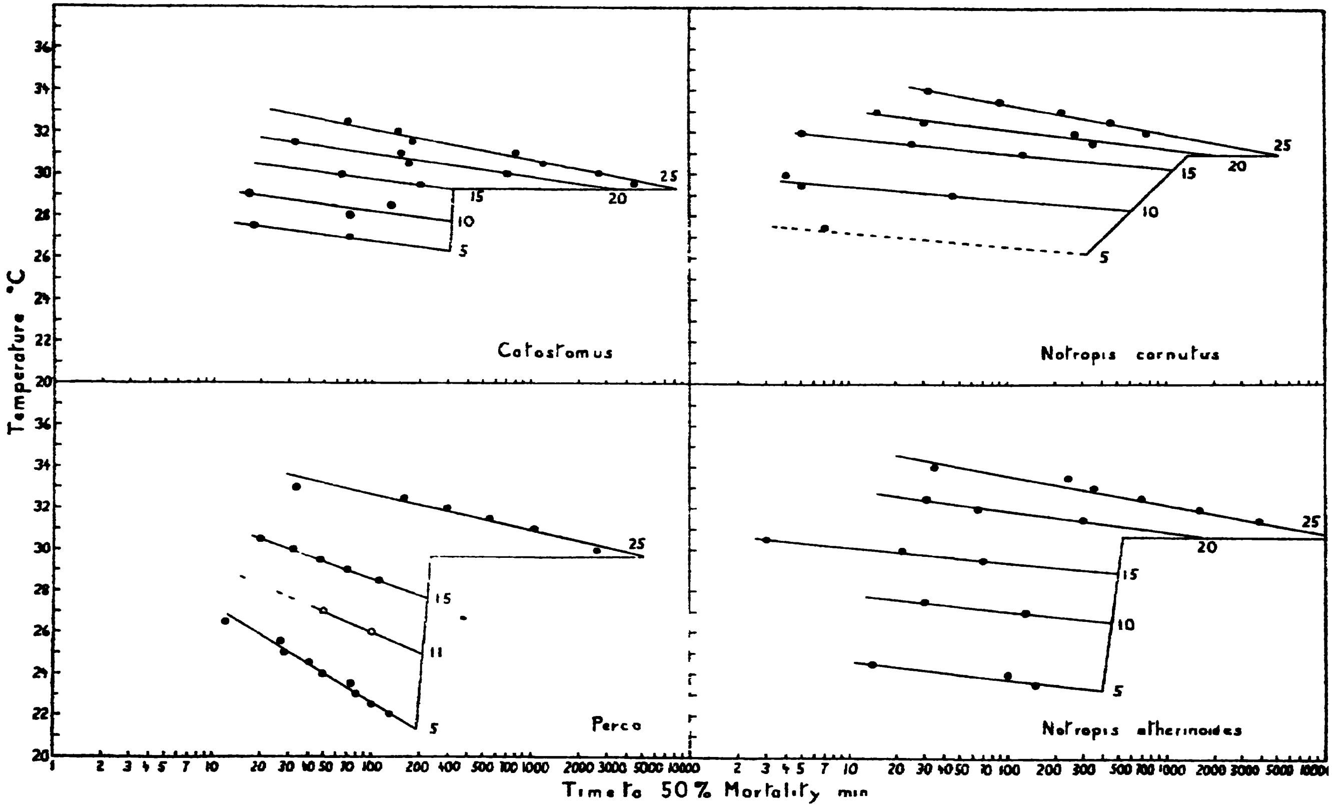


FIGURE 3.—The relation between temperature and time to death (resistance time) at various acclimation temperatures for *Catostomus*, *Perca*, *Notropis cornutus*, and *Notropis atherinoides*

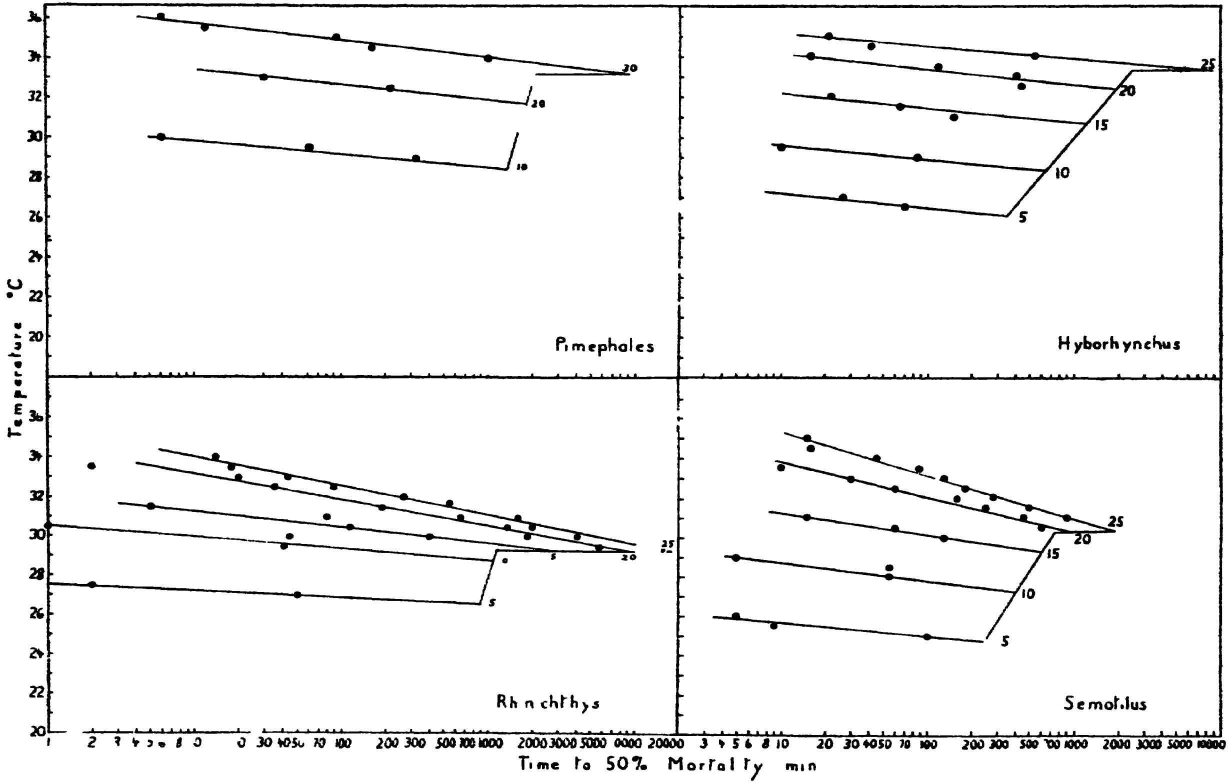


FIGURE 4.—The relation between temperature and time to death (resistance time) at various acclimation temperatures for *Hyborhynchus*, *Semotilus*, *Pimephales*, and *Rhinichthys*

of the fish species studied. Thus no further rise takes place in the incipient upper lethal of *Rhinichthys* after the acclimation temperature reaches and is increased beyond 11° C. There is thus a long "flat" portion to the curve. There are considerable variations in this respect, but there is a tendency for the "flat" portion to be reduced in species which can withstand the higher temperature levels.

(*c*) The change of lethal temperature for a given change in acclimation temperature. A rise of one degree in upper lethal temperature for a three degree rise in acclimation temperature was found previously for *Carassius*, *Ameiurus*, and *Girella*. The change for *Salvelinus* was much less, roughly 1:7. The changes found for *Perca* and *Notropis atherinoides* studied here were greater than any found so far, the former having a relative change of almost 3.5. Similar though not such extensive variations in this respect were observed for lower lethal temperatures.

2. *Thermal tolerance*

The thermal tolerance is the zone bounded by the upper and lower incipient lethal temperatures and can best be expressed according to the method of Fry *et al.* (1942) by measurement of the area contained by the trapezoid and is a measure of its eurythermicity. Table II indicates that the cyprinids are slightly more eurythermal than *Salvelinus* (625 square centigrade units) the most eurythermal being *Pimephales* (903), and the least, *Notropis atherinoides* (747). They do not approach *Carassius* (1,220 units) or *Ameiurus* (1,162) respectively (Brett, 1944).

3. *Thermal resistance*

As defined thermal resistance is a measure of the ability of a species to withstand temperatures above the level which will be finally lethal. The thermal resistance also determines when and at what temperature the fish will die in any changing temperature, the time and temperature at death being calculated from knowledge of the time to death at various constant temperatures above the incipient lethal level. This resistance time has been determined for each acclimation temperature and tabulated in Table III for eight species studied and for several species previously studied where data were available (Fry *et al.*, 1946; Doudoroff, 1942, 1945; Brett, 1944). The data have been plotted in Figs. 3 and 4.

In order to compare the resistance times of these species at a given acclimation level, the temperatures at which they would die when subjected to a rise in temperature of one degree per hour have been

TABLE III

Summary of resistance times. The values given are time to 50 per cent mortality in minutes based on a sample of five fish. A bracket indicates that only four fish died.

Species	*Accl level* °C	Test Temperatures °C																				Test Temperatures °C								
		22.0	22.5	23.0	23.5	24.0	24.5	25.0	25.5	26.0	26.5	27.0	27.5	28.0	28.5	29.0	29.5	30.0	30.5	31.0	31.5	32.0	32.5	33.0	33.5	34.0	34.5	35.0	35.3	36.0
Catostomus commersonnii	5											(70)	18																	
	10													70	130	16														
	15																(200)	63												
	20																	700	170	150	33									
	25																1500	2700	1200	800	180	115	70							
Rhinichthys atratulus	5											50	2																	
	10																40	45	1											
	15																	(400)	115	80	50									
	20																5700	1850	1335	750	190		35	20	3					
	25																	(4000)	2000	1600	550	265	90	43	18	14				
Semotilus atromaculatus	5							200	10	5																				
	10													55	56	5														
	15																	130	70	31										
	20																		600	450	250	160	60	30	10					
	25																			900	500	280	180	130	88	45	16	15		
Hyborhyncus notatus	5										70	26																		
	10															85	10													
	15																			150	65	22								
	20																						440	(400)	120	16				
	25																									550	42	21		
Pimephales promelas	10																60	6												
	20														300	320							220	30						
	30																									1000	160	92	12	6
Notropis cornutus	5												(7)																	
	10															45	5	2												
	15																			122	25	5								
	20																				350	270	30	15						
	25																					750	450	220	90	32				
Notropis atherinoides	5				150	100	24																							
	10											130	30																	
	15																(70)	22	2											
	20																				300	65	31							
	25																				(3800)	(1500)	700	350	245	35				
Perca flavescens	5	130	100	80	75	50	11	28	27		12																			
	11									50	100																			
	15														(110)	70	47	32	20											
	25																	(2500)		1050	550	300	160	33						

calculated, the rise taken as starting from the incipient lethal level according to the graphical method described by Fry *et al.* (1946). The 25° C. acclimation level was used in making these calculations. These results are given in Table IV.

TABLE IV

Comparison of the calculated death points of various species when exposed to a constantly rising temperature of one degree per hour. The temperature rise commences at the incipient upper lethal level for fish acclimated to 25°C.

Species	*Incipient upper lethal level* °C.	*Final temp. at death* °C.	*Difference final minus initial temp.*	*Time to death in minutes*
**Salvelinus fontinalis*. . .	25.3	29.0	3.7	222
Catostomus commersonnii . .	29.3	32.7	3.4	200
**Carassius auratus*. . . .	36.2	36.6	0.4	25
Rhinichthys atratulus .	29.3	33.7	3.4	202
Semotilus atromaculatus .	30.3	33.7	3.4	202
Hyborhynchus notatus .	33.3	34.8	1.5	90
Notropis cornutus.	31.0	34.0	3.0	180
Notropis atherinoides.	30.7	34.3	3.6	215
Perca flavescens.	29.7	33.4	3.7	220

*Data from Fry *et al.* (1946).

Table IV indicates that at the acclimation level of 25° C. most of the species studied withstand a rise in temperature of from 3° to 4° C. above their upper incipient lethal temperatures and most die in 180 to 222 minutes. Two of these, however, die quite soon (*Hyborhynchus* in ninety minutes and *Carassius* in twenty-five minutes) and do not withstand such a rise in temperature as the others.

The explanation of such variations in the survival time as are observed when different species are exposed to a changing temperature can be found in the resistance time pattern for those species. A description of the similarities and variations in these patterns will therefore be considered.

The description of the resistance time pattern given for *Salvelinus* emphasized several characteristics of resistance time which this species had in common with that of other species studied. With the additional knowledge at hand (Figs. 3 and 4), the general characteristics of the pattern have been found to include the following:

(*a*) The relation between the temperature to which the fish were exposed and the logarithm of the time to death is linear for a given acclimation temperature.

(*b*) The slopes of the resistance time lines for acclimation temperatures below the ultimate incipient upper lethal tend to be parallel but this is not always the case (Figs. 3 and 4). There is usually a marked change in slope for acclimation temperatures above the ultimate incipient lethal level.

(*c*) Although there is no increase in the incipient lethal temperature beyond a certain acclimation temperature, the resistance time has its greatest increase beyond this level. This follows from the fact that the time to kill a sample of fish at the incipient lethal temperature increases progressively as the acclimation temperature is increased (Table II).

The variations in resistance time pattern found in these species have been as follows:

(*a*) Large variations in the slopes of the resistance time lines (Figs. 3 and 4). Other things being equal, the greater slope makes for a greater ability to resist temperatures above the incipient lethal level. Thus the calculated death of *Hyborhynchus* (Table IV) was sooner than all the other species except *Carassius* largely by virtue of the fact that the slope at 25° C. was less than that of the other species. *Perca*, *Salvelinus*, *Carassius*, and *Semotilus* tended to have steeper resistance time lines than the other species.

(*b*) Large variations in the time to reach the incipient lethal temperature (Table III) ranging from less than thirty minutes in *Carassius* at 25° C. to about 20,000 minutes in *Rhinichthys*. The very low time in the case of *Carassius* accounts for the fact that the calculated survival was only twenty-five minutes (Table IV) in a rising temperature of 1° C. per hour.

CONCLUSIONS

The experiments described above confirm the work previously carried out in giving general expression to the way temperature acts as a lethal factor in fish species. The present study extends the scope of this work in pointing out the degree to which lethal temperature relations can vary between species.

Although the general pattern of thermal death is similar in the various fish, certain aspects of thermal death have been found here to

TABLE V

Size and age composition of the fish studied. Upper figures in each column represent numerical frequency in each weight class. Lower figures represent frequency in each age class

Species	Weight (gms.)	0-1.9	2.0-3.9	4.0-5.9	6.0-7.9	8.0-9.9	10.0-11.9	12.0-13.9	14.0-15.9	16.0-17.9	18.0-19.9	20.0-21.9	22.0-23.9	24.0 and over
	Age (years)	0	1	2	3	4	5	6	7	8	9			
Rhinichthys atratulus..	. .	83 (Age	93 not	6 determined										
Semotilus atromaculatus		26 6	66 59	23 27	9 7	14	8	11	2	5	3	0	3	3
Pimephales promelas		17	46 19	6 1										
Hyborhynchus notatus	..	75 11	15 36	1 5										
Notropis cornutus....	. .	3 2	19 11	39 42	25 19	6	4	4	5	5	4	2	2	1
Notropis atherinoides.	. .	147 58	22 11											
	Weight (gms.)	0-1.9	2.0-4.9	5.0-9.9	10.0-19.9	20.0-29.9	30.0-39.9	40.0-49.9	50.0-59.9	60.0-69.9	70.0-79.9	80.0-89.9	90.0-99.9	100 and over
Catostomus commersonnii .		5 4	17 26	44 26	50 8	8	4	3	1	2	1			
Perca flavescens... ..	. .				13 10	32 20	27 10	17 10	16 8	10 5	8 1	5	7	2

show even greater variations than was previously indicated. These variations include almost all the quantitative characters in the patterns of thermal resistance and incipient lethal levels. Certain of these were more extreme than was previously found.

It is not the purpose of the author to press the matter further at the present time. The data presented give a physiological description of several species and further conclusions at this point are not justified. Later work may assess the significance of the results presented here.

SUMMARY

Lethal temperature relations were determined over the whole range of thermal acclimation for eight species of fish from the Toronto region. The experiments involved the determination of (1) the upper and lower incipient lethal temperatures bounding the zone of tolerance (the range of survival) and (2) the upper thermal resistance time or the periods during which temperatures above those which will be ultimately lethal are withstood.

The tests extend the scope of the previous investigations in pointing out the diversity of the lethal temperature patterns. The comparative thermal data have been tabulated.

Thermal tolerance measurements indicated that the species here were in general intermediate in eurythermicity between *Salvelinus fontinalis* on the one hand (625 units) and *Carassius auratus* on the other (1,220 units). They ranged between 747 and 903 units. Upper ultimate incipient lethal temperatures tended to follow the degree of eurythermicity of the species and ranged from 29.3 to 33.3° C.

No correlation was found between size or age and the order of death at a given lethal temperature.

REFERENCES

BRETT, J. R. (1944). Some lethal temperature relations of Algonquin Park fishes. Univ. Tor. Stud., Biol. Series, no 52, Pub. Ont. Fish. Res. Laboratory, no. 63.

DEBEER, E. J. (1945). The calculation of biological assay results by graphic methods. The all-or-none type of response. J. Pharm. and Expt'l Therap., 85: 1-13.

DOUDOROFF, P. (1942). The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). Biol. Bull., 83: 219-44.

——— (1945). The resistance and acclimatization of marine fishes to temperature changes. II. Experiments with *Fundulus* and *Atherinops*. Biol. Bull., 88: 194-206.

FRY, F. E. J. (1947). Effects of the environment on animal activity. In press. Univ. Tor. Stud., Biol. Series, no. 55, Pub. Ont. Fish. Res. Laboratory, no. 68.

FRY, F. E. J., BRETT, J. R., and CLAWSON, G. H. (1942). Lethal limits of temperature for young goldfish. Rev. can. de biol., 1: 50-6.

FRY, F. E. J., HART, J. S., and WALKER, K. F. (1946). Lethal temperature relations for a sample of young speckled trout (*Salvelinus fontinalis*). Univ. Tor. Stud., Biol. Series, no. 54, Pub. Ont. Fish. Res. Laboratory, no. 66.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLI : SERIES III : MAY, 1947
SECTION V

Serious Mortalities in Prince Edward Island Oysters Caused by a Contagious Disease

By A. W. H. NEEDLER, F.R.S.C., and R. R. LOGIE

COMMENCING in 1915 a series of very severe mortalities, similar in every way, occurred among Prince Edward Island oysters. Starting with Malpeque Bay the mortalities eventually destroyed the fisheries in all the principal producing areas. The progressive nature of the mortalities and the absence of any marked changes in the physical environment are evidence of their being caused by a contagious disease. Experiments indicate that stock bred from the survivors of the epidemics in the areas first affected were resistant to the disease in its later outbreaks and that recovery in each inlet has taken place in the course of years in spite of the continued presence of the causative agent. It is suggested that a disease new to a highly susceptible Prince Edward Island oyster population was introduced, caused the severe mortalities, and remains present in a resistant population which has resulted.

The causative organism has not been determined but this account is presented because of the rarity of recorded cases of mortalities in the sea attributable to contagious disease. Many parasites and their activities have been described but few have been shown to be seriously pathogenic. It may, nevertheless, be surmised that diseases of this kind are not less common in the sea than on the land, and that they have not been as often demonstrated because of difficulties of observation and, perhaps, preoccupation with other factors. The few reported cases have been concerned with conspicuous and valuable species, for example eel-grass (*Zostera marina*) and herring (*Clupea harengus*). For an account of the severe mortality of eel-grass spreading over long distances on both sides of the North Atlantic see Renn (1936), and for a fungus disease of herring see Fish (1934).

Severe mortalities of oysters, of the same species as those in Prince Edward Island (*Ostrea virginica*), in several areas from Virginia to Louisiana, have been attributed by Prytherch (1938 and 1940) to the sporozoan *Nematopsis ostrearum* which has the mud crabs *Panopeus* and *Eurypanopeus* as primary hosts. *Nematopsis* has not yet been demonstrated in Prince Edward Island oysters, and the mortalities in the two regions appear to have somewhat different characteristics.

LOCALE

The oyster-producing areas of the Canadian Atlantic coast (Fig. 1) are limited to the southern part of the Gulf of St. Lawrence and the Bras d'Or Lakes in Cape Breton Island, except for the presence of a very few oysters in two widely separated and highly sheltered bays on the outer coast of Nova Scotia. Between the Canadian oyster populations and those of New England, which have their northern limit in the vicinity of Cape Cod, there are great distances with no oysters. The Canadian oyster populations constitute an isolated northern outpost.

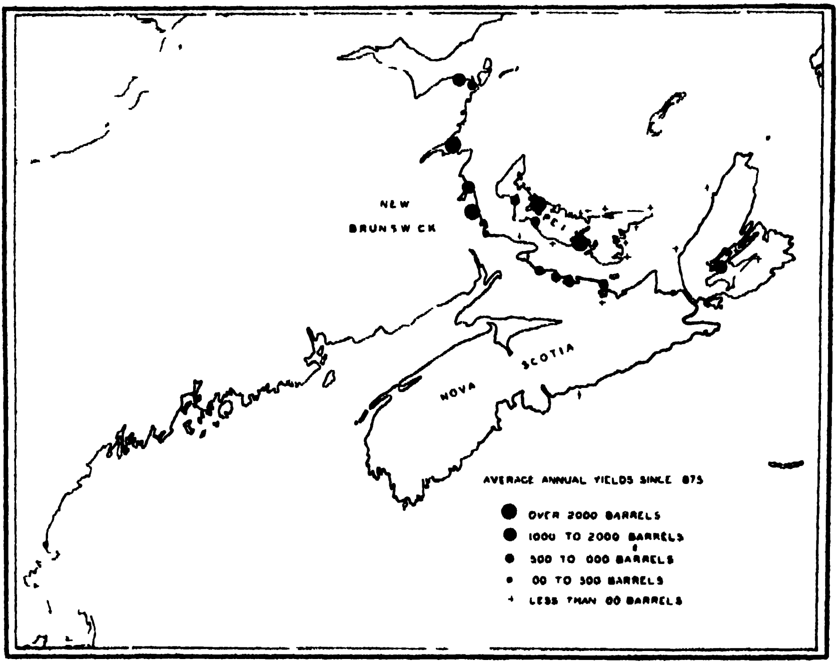


FIGURE 1.—Oyster-producing areas of the Maritime Provinces of Canada

Even in the southern part of the Gulf of St. Lawrence, which has higher surface temperatures than any other area on the Canadian Atlantic coast, the oysters are found only in the bays and the oyster populations of the individual bays are usually separated from one another by a number of miles. In Prince Edward Island (Fig. 2), Cascumpeque (*A*) and Malpeque (*B*) Bays are connected by sheltered water supporting oysters and may be considered a single oyster-producing area. The inlets of the Charlottetown vicinity—Hillsborough River

(*J*), York River (*K*), Elliot River (*L*), Pownal Bay (*H*) and Vernon, Orwell, and Seal Rivers (*G*)—are similarly a unit. So also are Percival and Enmore Rivers (*N*) and Brae Harbour (*O*). The oyster populations of the other inlets with which we are concerned are distinct.

CHARACTERISTICS OF THE MORTALITY

Oysters die of many causes—attack by starfish, drills, or other enemies smothering in silt or among other shellfish, over-long exposure to fresh water, etc.—and it is necessary to describe the characteristics setting the mortalities under discussion apart from the effects of the more ordinary factors.

The mortality with which we are concerned was very severe, usually affecting over 90 per cent of the original stock. In some cases it became difficult to find a single living oyster on grounds formerly well populated. Prior to the disease in the Malpeque area in 1915, no such severe mortalities had been recorded in Canadian Atlantic waters and records of fisheries preclude the possibility of their occurrence in any important producing area.

The epidemic affected oysters under a great variety of hydrographic conditions, over a number of years, in all types of bottom, and under varying degrees of crowding, whether by other oysters or other species such as mussels. Records of hydrographic conditions, quite complete during much of the outbreak, showed them to be well within the range known to be tolerated by normal oysters.

Symptoms included thinness and cessation of growth, weakness of the adductor muscle, failure to spawn, and the presence of yellow-green pustules. Oysters subject to the disease, when sampled, showed an unusually high proportion thin and translucent. Frequently the body shrank so as not to occupy the entire shell, limiting new shell deposition to a smaller area than previously. Growth was inhibited. Weakness of the adductor muscle was frequently shown by the ease with which affected oysters could be opened, and removal from the water commonly led to gaping and death much sooner than normally. Failure of oysters to spawn, although gonad development was well advanced, was common.

The most distinctive symptom associated with the disease was the frequent occurrence of yellow-green pustules. These contained amoebocytes and debris of the tissues in which they occurred. Although most common on the surface of the visceral mass, they occurred at times in a great variety of places, including the mantle (especially near its edge), the adductor muscle, and the heart. They were up to 0.5 cm. in diameter and size in general increased as the mortality approached its peak. Although of common occurrence in all affected stocks, oysters with the pustules sometimes did not appear in the samples examined until after some actual deaths had occurred.

The symptoms observed in Prince Edward Island oysters do not agree with those described by Prytherch (1940) and attributed to the gregarine *Nematopsis ostrearum*. He does not record the yellow-green pustules and indicates that general emaciation was not so common.

The death-rate varied seasonally, reaching a peak each year in the late summer and autumn

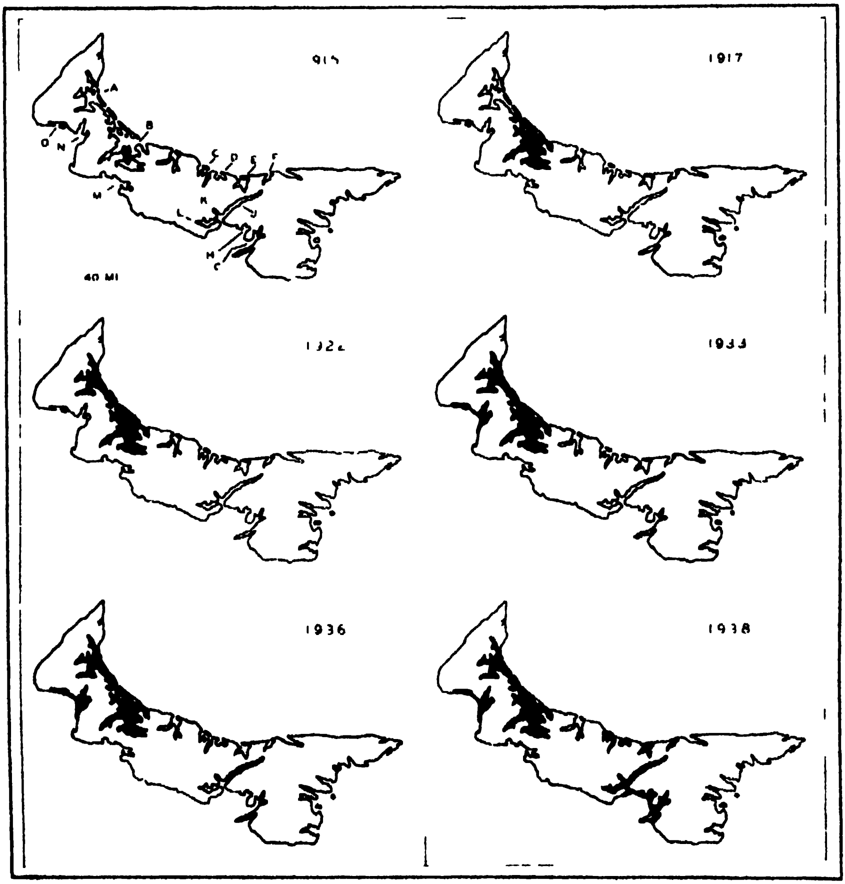


FIGURE 2—The spread of the oyster mortality. In each year the areas in which most of the oysters had died are blackened. *A*—Cascumpeque Bay; *B*—Malpeque Bay; *C*—Rustico Bay, *D*—Brackley-Covehead Bay; *E*—Tracadie Bay; *F*—Savage Harbour; *G*—Vernon, Orwell, and Seal Rivers; *H*—Pownal Bay, *J*—Hillsborough ("East") River; *K*—York ("North") River; *L*—Elliot ("West") River; *M*—Bedeque Bay; *N*—Percival and Enmore Rivers; *O*—Brae Harbour.

THE MORTALITIES AND THEIR SPREAD

Information on the earlier mortalities in the Malpeque-Cascumpeque area was obtained from the published reports of fisheries officials, from the members of the industry who had observed them, and from the published statistics of the fishery. To these sources were added, from 1929 on, our own observations.

Malpeque-Cascumpeque area, 1915-21

The early history of the oyster industry in this area has already been described (Needler, 1931). In brief, an intensive fishery reduced earlier higher yields to a level of about five thousand barrels annually about which the yields varied for about a decade before oyster farming, which had been started on a very small scale before 1900, was encouraged by the leasing of oyster ground by the provincial government in 1913. This policy led to the first considerable plantings of small oysters from the United States in 1914.

In 1915 large numbers of oysters were found dead or dying in the central part of Malpeque Bay, in the area where the imported oysters had been planted In 1916 the mortality spread throughout the bay and its immediate tributaries.

Mr. G. S. Sharp, an oyster farmer who had been active for many years in the western part of the bay, stated that survey of his beds in July led him to estimate that his crop in November would be about one thousand barrels. By that time, however, so many of the oysters had died that he was only able to fish a few dozen barrels of living oysters, most of which died soon after being taken from the water. Those he did obtain were thin, watery, and had yellow-green pustules. Others who were in the industry at that time confirm the occurrence of the heavy mortality in the late summer and autumn, the poor condition and keeping qualities of the survivors, and the presence of yellow-green pustules.

The epidemic spread through the oyster population of the sheltered waters inside the barrier beach to Cascumpeque Bay and its tributaries, affecting the eastern-most tributary in 1918 and the western and northern tributaries in 1920 and 1921. By the end of 1922 the severe mortality had covered the entire Malpeque-Cascumpeque area, and no oysters were obtained by the commercial fishery in that year.

The mortality in the Malpeque-Cascumpeque area is reflected in the statistics of the oyster fishery in Prince County, P.E.I. (Fig. 3). Unfortunately this statistical area included inlets on the south coast having small oyster fisheries, but the annual yields show the effect of the disappearance of the commercial fishery from the principal producing area of the county.

Enmore-Percival area, 1933

The fishery in this area, including Brae Harbour, has never exceeded a few hundred barrels and in many cases, as the oysters were landed by the same fishermen and shipped by the same packers as those from the neighbouring Malpeque-Cascumpeque area, the published statistics give little indication of its changes. Local report indicates, however, that the fishery had for many years been at a low and fluctuating

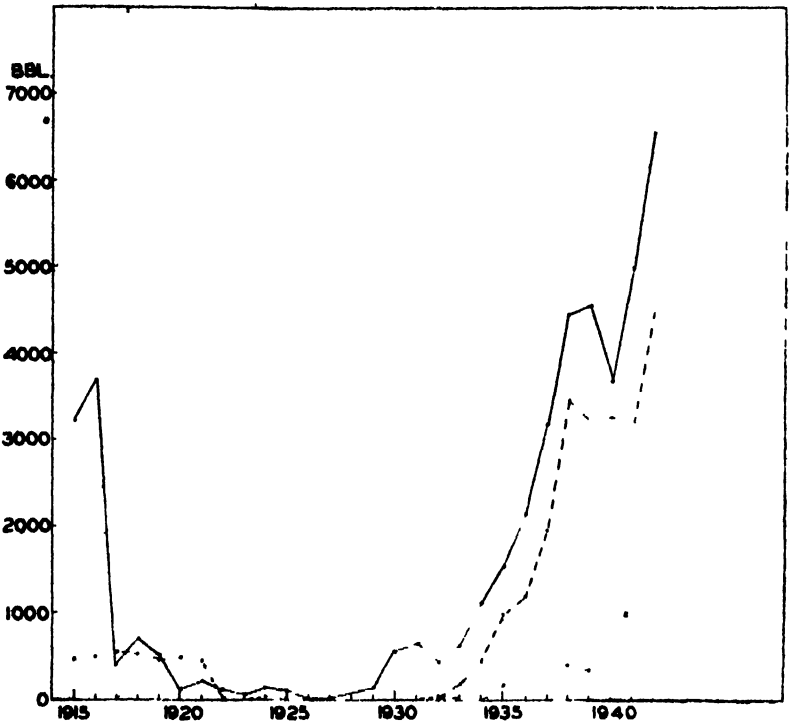


FIGURE 3—Annual yields of the oyster fishery in Prince County, P.E I. Yields in the eastern and western parts listed in the published statistics are shown by the solid and broken lines respectively. The eastern part includes the Malpeque Bay area, which is the principal producer, but also the Bedeque and the Enmore-Percival areas on the south coast. The western part includes the Cascumpeque Bay area and some small production on the south coast at Brae Harbour. The dash-and-dot line shows the production by oyster farming in the Malpeque Bay area as given by statutory declarations by lessees of oyster ground.

level but had continued until 1932. In 1933 the fishery was a complete failure.

Many of the fishermen had been working, using the same boats and gear, on oyster farms in the Malpeque area earlier in the same year. As detailed below, introductions of Enmore oysters into the Malpeque area in 1924 to 1929 had shown their susceptibility to the disease. The fishermen may have carried the disease to the Enmore area either with gear or with small oysters or some other host of the causative organism.

An attempt in 1934, under the senior author's supervision, to reestablish the Enmore River fishery by transferring three hundred barrels of small oysters from the intertidal zone, where they were exposed to winter killing, to deeper water met with failure. In the late summer of 1935 the few survivors which could be found alive were in poor condition and some had characteristic yellow-green pustules.

Charlottetown area, 1935-9

The Charlottetown area, including Hillsborough, York, Elliot, Vernon, Orwell, and Seal Rivers and Pownal Bay, had always had a considerable production of oysters. After the disappearance of the eelgrass from a contagious disease in 1931, which made finding oysters on the extensive tidal flats of the area much easier, the production rose to about eight thousand barrels, Hillsborough River contributing the greatest share.

In 1935 small oysters were dredged in the upper part of Hillsborough River for sale to oyster farmers in neighbouring bays on the north shore of the province. In the early summer of 1936 the experimental collection of spat proceeded normally. Neither of these operations gave evidence of a serious mortality. In the autumn of 1936, however, the fishery in Hillsborough River was a failure, with a consequent reduction in the total catch of the area (Fig. 4). Survivors examined in October showed poor condition and characteristic yellow-green pustules.

The fishery in York and Elliot Rivers was not affected in 1936, the serious mortality being limited to Hillsborough River. Oyster fishermen who had worked in the Malpeque area earlier in the season first came to the Charlottetown area in 1934 and 1935, making their headquarters in Hillsborough River near the centre of the area affected by the mortality in 1936. Fishermen may have brought the disease to Hillsborough River from Malpeque, as they may have to Enmore River. A transfer of Hillsborough River oysters to Malpeque Bay in 1928 had shown their susceptibilty to the disease (see below).

The disease spread in 1937 to all other inlets in the area and continued in 1938. Hardly any oysters of marketable size could be found in 1939 and the fishery practically disappeared (Fig. 4). The mortality spread from Hillsborough River, affecting York and Elliot Rivers (40 to 75 per cent mortality in 1937) before Pownal Bay and Vernon and Orwell Rivers (10 to 35 per cent in 1937). In all these inlets the mortality among adult oysters was well over 90 per cent of the original stock by the end of 1938, as judged by the proportion of living oysters to newly empty shells and by the numbers which could be found before and after the outbreak.

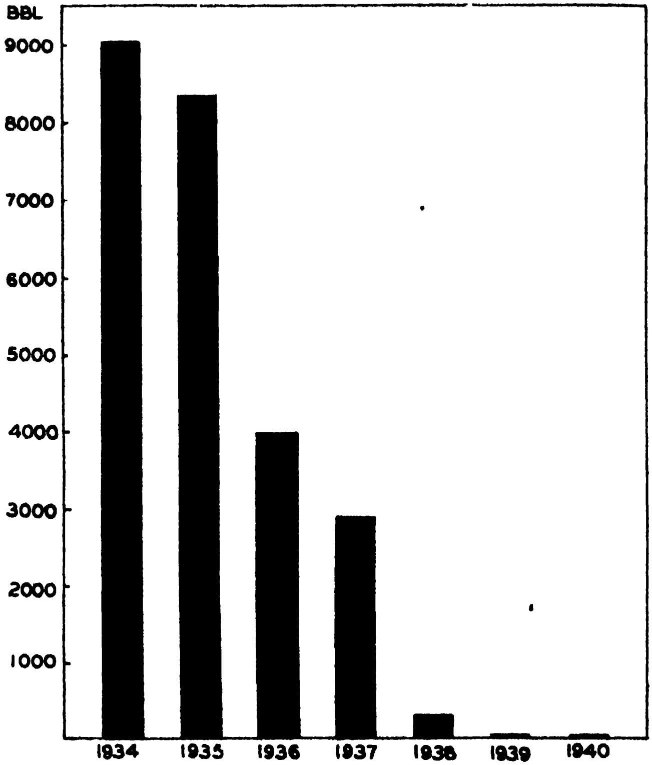


FIGURE 4—Annual yields of the oyster fishery in Queens County, P.E.I., taken almost entirely in the Charlottetown area

Oysters were held under observation at ten points in this group of inlets in 1937 and 1938 and samples were frequently examined. In some cases it was difficult to find as many as one hundred living oysters of marketable size for this purpose, and these survivors continued to die at an abnormally high rate, frequently exceeding 50 per cent in the single season under observation. Large numbers of younger oysters and of currently produced spat were also sampled and examined. From these observations it was found (1) that the serious mortality commenced usually about the beginning of August and rose to a maximum in the autumn, (2) that frequently it commenced before oysters with characteristic pustules were found in the samples examined, (3) that surviving adult oysters in the region had very poor average growth and condition, and (4) that production of oyster spat continued but that they suffered considerable mortalities (see also the experiments below).

North-shore bays near Charlottetown, 1936-8

In Rustico, Brackley-Covehead, Tracadie, and Savage Bays former oyster populations had long been reduced to a very low level principally, it is believed, by the increased silting caused by intensive agriculture. In 1935 there had been no commercial production in these inlets for many years, but prospects for oyster farming seemed reasonably good and about 450 barrels of small oysters from Hillsborough River were planted on leased areas. These were examined in 1936 and the following years and serious mortalities occurred in all instances, there being few survivors of the transferred oysters by the end of 1938. The mortalities occurred in the Hillsborough River oysters first but native oysters were also affected, a heavy mortality commencing in 1937 among a considerable native population in a small tributary of Rustico Bay.

By 1939 living adult oysters, whether of Hillsborough River or native stock, were difficult to find in all of these inlets. The characteristic seasonal occurrence of the mortality, yellow-green pustules, and poor condition were observed as in the Malpeque-Cascumpeque, Enmore, and Charlottetown areas, and it is probable that the disease was carried to these north-shore bays with the oysters transferred from Hillsborough River.

Other Prince Edward Island areas

In a number of other inlets with very small oyster stocks our information on the occurrence of the mortality is less definite. Introductions of stock from the Malpeque-Cascumpeque or Charlottetown areas have been widespread in attempts to establish oyster farming. In all known cases Malpeque stock has been unaffected by a mortality of the kind under discussion, and Charlottetown stock transferred in 1935 or later has suffered it. In Pinette River, a small inlet east of Vernon and Orwell Rivers, planted Charlottetown stock and native stock both suffered a typical heavy mortality in 1937 and 1938.

In Bedeque Bay the picture is uncertain. The published statistics do not distinguish between shipments of oysters from Bedeque and Malpeque Bays, and local reports are confused. It seems, however, that production from this bay disappeared at about the same time as that from Malpeque Bay. As the same people in many instances fished in both bays it is probable that this decline is attributable to introduction of the same disease. This is confirmed by experimental transfers which have shown that oysters now occurring in Bedeque Bay have the same resistance to the mortality as Malpeque oysters (see section below on development of resistant stocks).

Summary

A very severe mortality commencing in Malpeque Bay in 1915 has now affected all the oyster stocks in Prince Edward Island on which definite information is available. In all cases of spread to unaffected, separate inlets a mechanism for transfer by land is apparent, either through known transfers of oysters or through transfers of gear and boats which might carry oysters or other hosts of a causative organism. The mortality has everywhere had the same seasonal occurrence and been accompanied by the same poor condition among survivors and by the appearance of similar yellow-green pustules on some of the oysters.

There has been no evidence of the occurrence of a similar mortality in any of the oyster populations of Nova Scotia or New Brunswick.

RECOVERY OF OYSTER POPULATIONS

The recovery of oyster populations in areas affected by the mortality has shown a characteristic pattern. In all cases production of spat has continued throughout and following the mortality, especially at the heads of inlets where high temperatures normally lead to the best reproduction in this region (Needler, 1941). Consecutive broods of young oysters have shown high mortalities at first but better and better survival until the mortality falls to a normal level. This declining mortality rate has been reported by all observers.

In the Malpeque area the mortality first occurred in 1915 and 1916. The recurrent "sets" of spat led to unjustified hopes for a rapid recovery. In the earlier years few survived to marketable size. Fishing was not restricted but no oysters were taken until 1925 when it is recorded that four barrels were fished, to be followed by fourteen barrels in 1926. Recovery was slow but the stock was sufficient to serve as the basis for the oyster farming development shown in Fig. 3. Mortalities among oyster spat reared experimentally from 1929 onwards were low (less than 10 per cent), except when some other factor was definitely in evidence (such as attack by starfish, etc.). In this area survival of oysters appeared to be restored to normal in not more than thirteen years from the original mortality. The causative agent remained present, however, as indicated by the apparent transfer of the disease to the Charlottetown area as late as 1934 or 1935, and by the death of oysters introduced from unaffected areas as late as 1929 (see below).

In the Charlottetown area there are at the time of writing (about eleven years after the first heavy mortality in Hillsborough River) considerable quantities of oysters of marketable size present on some grounds. As noted above, the same recurrent broods of spat were produced with a mortality which was high at first and has since declined.

Development of Resistant Stocks

Evidence of the development of resistant stocks is of two kinds: (1) the recovery of Malpeque stocks in spite of the continued presence of the disease as indicated by the death of oysters introduced from unaffected areas; (2) the good survival of Malpeque oysters in affected areas as compared with that of native stock.

Introduction of oysters into the Malpeque area from unaffected areas, 1924-9

The first introduction of oysters into the Malpeque area after the mortality of which we have knowledge was made by Mr. G. S. Sharp who planted oysters from Enmore River on his private area in 1924. He reported that dying oysters with the same appearance as in the original mortality were found in 1925 and that no survivors could be found in 1926.

In 1928 the Department of Fisheries, in an attempt to accelerate recovery in the Malpeque area and to demonstrate oyster farming methods, planted seventy barrels of marketable oysters from Hillsborough River and about thirty barrels of yearlings from Enmore River. When examined early in 1929 they appeared to be in good condition and had commenced growth since planting. When examined by the senior author in late October, 1929, and again in February, 1930, they were in poor condition, about one-third of the Hillsborough River oysters showing the yellow-green pustules since found to be typical, and one being found so recently dead that the meat remained present. An intensive attempt to fish these oysters in 1930 succeeded in recovering only two or three barrels alive.

The Enmore River oysters, which were younger, fared slightly better but only five barrels could be recovered in June, 1930, whereas normal growth would have produced close to one hundred barrels.

In October, 1929, to establish a spawning stock for observation, thirty-seven barrels of Enmore River oysters of mixed sizes were planted on a small patch of excellent bottom and twenty-three barrels on another which was somewhat softer bottom but, nevertheless, satisfactory. In the spring of 1930 survival was good and both development of the gonads and growth made a normal start. Growth soon slowed, condition became poor, spawning was abnormally delayed and incomplete, and yellow-green pustules appeared. By November there was considerable difficulty in procuring a sample of a hundred for examination and in 1931 no survivors could be found. Adjacent Malpeque stock showed no unusual mortality.

These introductions of oysters from Hillsborough and Enmore Rivers indicate: (1) that the agent causing the mortality was still present, (2) that the oysters from Hillsborough and Enmore Rivers were highly susceptible, (3) that mortalities over 90 per cent and, in the last case, approaching 100 per cent were caused, and (4) that, as in the case of native stocks, the mortality occurred principally in the late summer and autumn and was accompanied by poor condition and the occurrence of yellow-green pustules in some of the survivors. In spite of the continued presence of the causative agent native Malpeque stock showed no serious mortalities.

Among many hundreds of native Malpeque oysters examined in the course of these and other investigations in 1930 and 1931, only a very few (estimated at about one per thousand) showed any sign of the yellow-green pustules associated with the disease, and they did not reach the sizes common in diseased stocks. This suggests that, the causative agent being present, mild cases of the disease continued to occur in the native Malpeque stock.

Transfers of Malpeque stock to affected areas

Because of the apparent resistance of Malpeque stock to the disease, its transfer to affected areas was carried out experimentally and its use by prospective oyster farmers encouraged. The plantings of Malpeque oysters met with the usual varying success, dependent on the suitability of bottom conditions, etc., but in no recorded case did they show mortalities with symptoms typical of the disease, and when held under the same conditions as native stock they invariably showed better survival. The factors governing the survival of planted oysters are so complex that accurately controlled experiments were not possible. It may, however, be stated that, in numerous transfers to areas affected by the disease, Malpeque oysters showed no evidence of being affected while native oysters held under the same conditions showed both higher mortalities and typical symptoms.

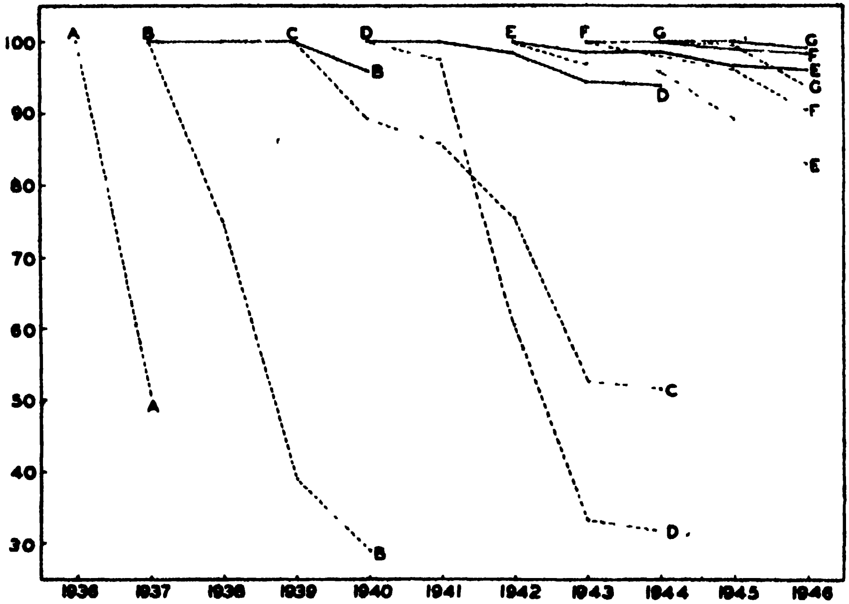


FIGURE 5.—Percentages of Malpeque and native oyster spat surviving on trays at Enmore River Malpeque spat shown by solid and Enmore spat by broken lines *A* are spat of 1936, *B* of 1937, *C* of 1939, *D* of 1940, *E* of 1942, *F* of 1943, *G* of 1944.

Experimental rearing of Malpeque and native spat in affected areas

In a number of experiments Malpeque spat were reared with native spat in both Enmore and Hillsborough Rivers. The spat were obtained on concrete-coated cardboard collectors and reared on the wire-bottomed floating trays developed in this region as a standard oyster cultural method (Needler, 1941). In these experiments the conditions under which the Malpeque and the native spat were held were kept as similar as possible but there are many physical difficulties, such as possible smothering when lowered to the bottom over winter and differences in crowding arising from different rates of survival and growth. The results are, however, so consistent that certain conclusions may be drawn safely. Mortalities definitely attributable to factors other than the disease (such as loss by breakage of trays, etc.) are not included in the calculated percentage survivals shown in Figs. 5 and 6 for the experiments in Enmore and Hillsborough Rivers respectively.

In all cases the survival of the Malpeque spat was greater than that of the native spat of the same year-class. The survival of Malpeque spat was normal and, in fact, would be considered excellent with the exception of the 1940 year-class at Hillsborough River where the conditions were frequently poor because of bad silting. The native spat, on the other hand, showed high mortalities.

At Enmore the native spat of the year-classes of 1936, 1937, 1939, and 1940 had poorer survivals than the later year-classes (Fig. 5). This accords with the observed mortalities among the recurrent broods of small oysters during the first few years after the appearance of the serious mortalities in the Malpeque and Charlottetown areas. As noted above, the survivals became normal in the Malpeque area in not more than thirteen years after the original mortalities. The first severe mortality in Enmore River was in 1933 and thirteen years later the survivals appear to be approaching normal. In the Hillsborough River experiments (Fig. 6) there is somewhat less indication of the decline in mortality but the original mortality was more recent there (1936).

The mortalities of the native stock in these experiments were accompanied by occurrence of yellow-green pustules and by poor condition and slow growth. Measurements showed the slower growth of the native spat as compared with the Malpeque. For example, Enmore spat of the year 1937 reached an average weight of 81 gm., as compared with 118 gm. for the Malpeque spat, although the latter were slightly smaller at the beginning of the experiment.

CONCLUSIONS

1. The severe mortalities among Prince Edward Island oysters are believed to have been caused by a contagious disease because (1) the severity was unprecedented and occurred over a considerable period without any accompanying abnormal physical changes in the environment; (2) the mortalities in the two major areas spread outward from the starting point to cover the whole area in a few years; (3) the mortalities spread throughout these continuous oyster populations steadily from the time of their appearance but spread to separate populations only when there was evident means of transfer by land; and (4) the characteristics of the mortalities were everywhere similar.

2. The mortality was severe (over 90 per cent) and recovery slow in all cases studied. During and after the incidence of the disease spat and small oysters continued to be produced. At first these broods themselves suffered severe mortalities, but survival improved and became normal in the Malpeque region in not more than thirteen years.

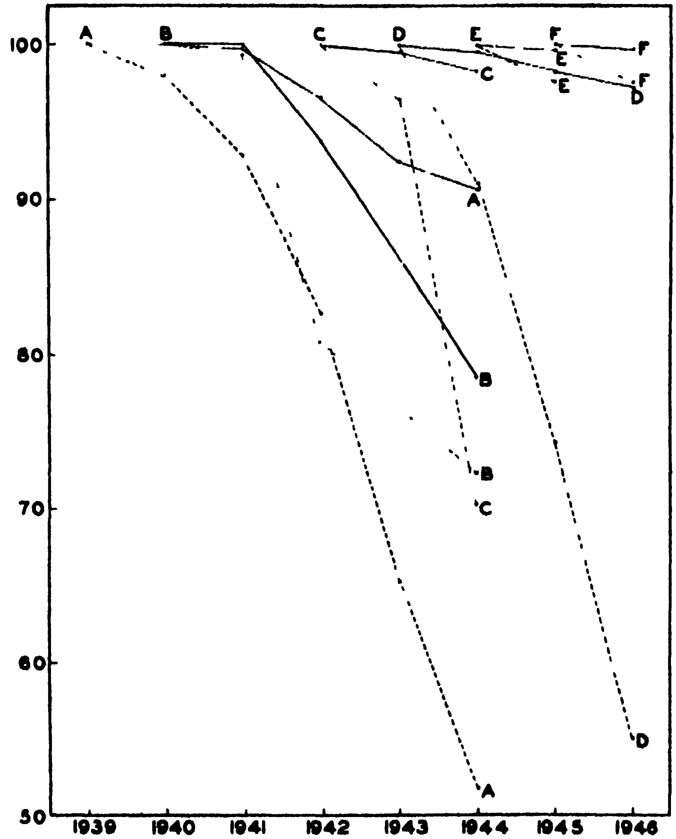


FIGURE 6—Percentages of Malpeque and native oyster spat surviving on trays at Hillsborough River. Malpeque spat shown by solid and Hillsborough River spat by broken lines *A* are spat of 1939, *B* of 1940, *C* of 1942, *D* of 1943, *E* of 1944, *F* of 1945.

3. The continued presence of the disease in the Malpeque area after the survival of the local stock became normal, is shown by communication of the disease to introduced oysters in every case within a year of introduction, resulting in the typical symptoms and very high mortalities.

4. The development of resistance to the disease is shown by (1) the recovery in the Malpeque Bay area in spite of the continued presence of the disease, and (2) the normal survival of Malpeque spat reared on trays in affected areas with native spat showing high mortalities and typical symptoms. In the many plantings of adult Malpeque oysters in affected areas there has been no evidence of mortality caused by the disease.

5. The severity of the mortality suggests that the disease was new to the isolated Prince Edward Island population. A possible source is offered by the introduction of oysters into Malpeque Bay from New England, which first took place on a large scale just before the appearance of the disease. The reported absence of any similar mortalities in the source area at that time might be explained by resistance of the stock there to the disease.

6. As the disease has affected all the principal Prince Edward Island oyster areas, and as there is great danger of its introduction to any small producing areas which it may not yet have reached, it is concluded that it would be unwise to base oyster farming anywhere in the province on susceptible stock, and the use of stock from areas already affected by the disease should be encouraged everywhere within the province.

7. As there is no indication that the disease has occurred in other parts of the isolated Canadian oyster population, and as the stock in Nova Scotia and New Brunswick may therefore be susceptible it is concluded that, in order to protect the valuable oyster fisheries there from the same interruption over many years which occurred in Prince Edward Island, every effort should be continued to avoid transmitting the disease to areas outside the province. As there is a suggestion that the disease was carried to the Charlottetown and Enmore areas by transport of gear and boats used in the oyster industry in the Malpeque area (with or without oysters or other hosts of the causative organism), care should be taken to avoid using Prince Edward Island oyster fishing or farming equipment outside the province.

8. It is suggested that the susceptibility of the stocks in important oyster areas outside the province might be tested by small-scale experimental introductions to affected areas in Prince Edward Island.

ACKNOWLEDGMENTS

The oyster mortalities in Prince Edward Island were studied as part of the Fisheries Research Board's investigations associated with the oyster farming program of the Department of Fisheries of Canada.

Various other members of the Board's staff have shared in the work. Dr. C. J. Kerswill was in general charge from 1941 to 1945 and supervised the experimental rearing of Malpeque and native oysters in affected areas. Mr. R. E. S. Homans observed the course of the mortality in 1937, 1938, and 1939 in the Charlottetown region and assisted with experimental transfers of Malpeque oysters to affected areas. Dr. A. B. Needler observed the course of the disease in Enmore River oysters transferred to the Malpeque area in 1929. Professor Roy Fraser examined diseased oysters bacteriologically and histologically at Mount Allison University in 1937-8. Dr. L. R. Richardson of McGill University examined diseased oysters in the field in the summers of 1938 and 1939.

REFERENCES

Fish, F. F. (1934) A fungus disease in fishes of the Gulf of Maine Parasitology, **26**· 1-16.

Needler, A. W. H (1931). The oysters of Malpeque Bay Biol Board of Canada, Bull 22 1-30

— —(1941) Oyster farming in Eastern Canada Fish Res Board of Canada, Bull 60: 1-83

Prytherch, H. F (1938) Life cycle of a sporozoan parasite of the oyster. Science, **88**. 451-2

———(1940) The life cycle and morphology of *Nematopsis ostrearum*, sp. nov, a gregarine parasite of the mud crab and oyster J Morph, **66** (1) 39-67.

Renn, C E (1936) The wasting disease of *Zostera marina* Biol Bull, **20**: 148-58

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLI : SERIES III : MAY, 1947
SECTION FIVE

The Deaminase Activity of *Proteus Ichthyosmius*

By NORA E. NEILSON and BLYTHE A. EAGLES
Presented by A. H. HUTCHINSON, F.R.S.C.

CONSIDERABLE data are available on the deamination of amino acids by various bacterial species, but little attention has been given to the deaminating activities of members of the genus Proteus.

The present study was undertaken with the object of determining the conditions governing the deamination of amino acids by resting cell suspensions of *Proteus ichthyosmius*.

EXPERIMENTAL METHODS

The micro-organism employed in this study was *Proteus ichthyosmius* (Hammer).

Preliminary investigations had shown that *Proteus ichthyosmius* attacked several amino acids with considerable vigour. In the light of this survey and in view of the interesting structural relationships between certain amino acids as postulated by Krebs (1939), the following amino acids were selected for detailed study: aspartic acid, glutamic acid, arginine, histidine, and proline.

Washed cell suspensions were prepared by growing the culture in a thin layer of tryptic casein digest broth in a Roux flask for twelve hours at 37° C. Following incubation, the cells were centrifuged and washed once with a phosphate buffer of M/30 concentration at pH 7.4.

The deaminase activity of the micro-organism was determined by incubating the washed cell suspension with the amino acid in a buffer solution. The amino acid was employed at M/200 concentration, the buffer at M/30, and the bacterial cells at a concentration twice that of maximum growth in the broth medium. Of this mixture 30 ml. were incubated at 37° C. in a 125 ml. Erlenmeyer flask; 5 ml. aliquots were removed at definite intervals and the ammonia content was determined by the Van Slyke procedure.

Deaminase activity was measured at pH 5.5, 6.5, 7.5, and 8.5. In the case of *l*-histidine, pH 9.5 was also employed.

The endogenous ammonia nitrogen was determined by incubating the cell suspension in the buffer solution in the absence of the amino acid substrate and in the calculation of the results this value has been subtracted from the total ammonia nitrogen formed in the presence of the respective substrates

The results thus obtained are expressed as ammonia nitrogen formed in per cent of the total nitrogen of the amino acid in the reacting suspension and are given in Table I

TABLE I

DEAMINASE ACTIVITY OF *Proteus ichthyosmius*
(Ammonia nitrogen formed in per cent
of total nitrogen)

Amino Acid	*Time* (hours)	pH 5 5	pH 6 5	pH 7 5	pH 8 5
dl aspartic acid	½	12	38	38	38
	1½	40	40	40	40
l aspartic acid	½	92	84	88	80
	1½	72	76	76	70
d glutamic acid	2	6	14	20	16
	4	10	28	36	28
	8	16	54	60	44
l glutamic acid	2	8	16	20	16
	4	10	30	36	28
	8	16	56	58	46
l-histidine	1	3 3	4 6	7 3	8 6
	2		7 3	12 6	
	4	5 3	10 0	19 3	24 6
	6		15 3	28 6	
	8	8 6	20 0	32 6	34 7
l-proline	1	6	12	12	12
	2		16	20	
	4	20	26	32	28
	6		38	44	
	8	38	40	52	34
l arginine	1		43 5	45 5	
	2	45	48 5	45 5	47
	4			52	
	6	47 5	50	48	41 5
d-arginine	1		42 5	48 5	
	2	45	50	51	42 5
	4		51 5	52	
	6	49 5		50 5	42 5

DISCUSSION

In the case of aspartic acid, the results show that the *l*-isomer is completely deaminated by *Proteus ichthyosmius* and indicate that the *d*-form is not attacked. The rate of deamination is rapid, the maximum value being reached within thirty minutes' incubation. The enzyme would appear to be uniformly active over the pH range 5.5 to 8.5.

In contrast with the findings obtained for aspartic acid, both isomers of glutamic acid are equally deaminated, but the rate of deamination is relatively slower. Although this reaction occurs over a wide range of pH, a definite optimum is shown at pH 7.5. The results suggest that in the case of glutamic acid the deaminase enzyme system is adaptive in nature.

l-Histidine is deaminated over the range of pH studied, but a definite optimum is evident at pH 8.5. The results show that one-third of the total nitrogen is liberated, suggesting that the alpha amino group undergoes complete deamination after eight hours' incubation. Although in the case of this organism there is no direct evidence that the iminazol ring is opened, this possibility must be entertained until conclusive proof is forthcoming that the alpha amino group is the only nitrogenous group attacked.

l-Proline is also deaminated over the range of pH studied, but the optimum is shown to be at pH 7.5. The pyrollidine ring is opened by *Proteus ichthyosmius* and the compound thus formed undergoes fairly rapid deamination. In preliminary studies on the influence of oxygen tension on this reaction, evidence has been obtained to suggest that the opening of the ring is oxidative in nature.

Both the *l*- and *d*-isomers of arginine are attacked with equal vigour at all pH levels studied without showing evidence of a characteristic optimum pH. Fifty per cent of the nitrogen is liberated in the form of ammonia. No evidence of urease activity by *Proteus ichthyosmius* could be demonstrated, thus confirming the findings of Rustigian (1941). Also, no evidence of urea formation from arginine could be obtained. The findings thus suggest that the mode of attack of arginine by *Proteus ichthyosmius* resembles that postulated by Hills (1940). The enzyme is considered to be an "arginine dihydrolase" rather than an "arginase" in the accepted sense.

SUMMARY

The conditions governing the deamination of amino acids by resting cell suspensions of *Proteus ichthyosmius* (Hammer) have been studied and preliminary findings are reported. Considerable variation in the ability to attack different amino acids was observed. Whereas *l*-aspartic acid was practically completely deaminated in one-half hour, *d*-aspartic acid did not appear to be attacked. In the case of glutamic acid, both isomers underwent slow deamination,

suggesting an adaptive deaminating enzyme system. With *l*-histidine evidence was obtained that at least one nitrogen was completely deaminated, probably the alpha amino group of the side chain. The nitrogen of the imino group of *l*-proline was liberated slowly as ammonia through the opening of the pyrollidine ring. Both the *d*- and *l*-isomers of arginine were decomposed, yielding at least two of the nitrogens in the form of ammonia. The reaction process is shown to be similar to the action of arginine dihydrolase.

UNIVERSITY OF BRITISH COLUMBIA

REFERENCES

HILLS, G. M. (1940). Biochem. J., **34**: 1057.
KREBS, H. A. (1939). Enzymologia, **7**: 53.
RUSHIGIAN, R. and STUART, C. A. (1941). Proc. Soc. Expt'l Biol. and Med., **47**: 108.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLI : SERIES III : MAY, 1947
SECTION FIVE

Variations of Thermogenesis during the First Stages of Seed Germination

By HENRI PRAT

Presented by JACQUES ROUSSEAU, F.R.S.C.

THE experiments were performed with the microcalorimeter of Tian and Calvet. This apparatus gives a direct measurement of the thermic flux, i.e. the quantity of heat produced during the time-unity. Its high sensibility detects heat emissions as small as 0.01 calories in an hour. By means of a system of thermo-electric couples the thermogenesis curve is directly obtained, by continuous record of a galvanometer spot on a revolving drum.

Starting from the moment when water is put in contact with the seeds, we can distinguish on the curve three consecutive phases: (1) a sudden outflow of heat, corresponding to hydration of the dry matter: *physico-chemical thermogenesis* (*OAB*, Fig. 1) ; (2) a time of latence, where thermogenesis fades out, or even can be replaced by an absorption of heat (*BC*, Fig. 1) ; (3) a new, slow uprising of the heat production, representing the real start of germinative process, with intervention of an increasing intensity of respiration: *biological thermogenesis (CD*, Fig. 1).

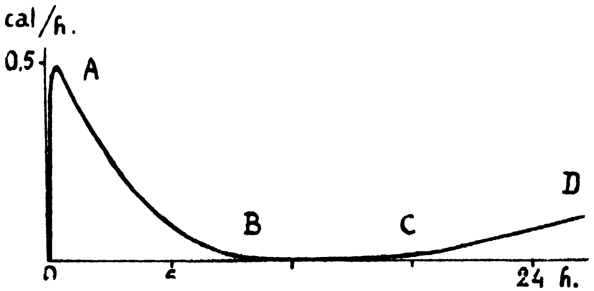


FIGURE 1.—Example of thermogenesis curve for wheat grains (1 gram, 17°C.). *OAB* physico-chemical thermogenesis; *BC* time of latence, *CD*: biological thermogenesis.

In all cases we find the same periods, but their durations and intensities are different according to the species. For instance, for wheat grains, at 17° C., the first stage lasts about ten hours and the third begins about eighteen hours after the moment of soaking (Fig. 1). For turnip seeds, the first stage requires only one hour, and the third stage begins three or four hours only after moistening (Fig. 2).

Intensities also are different. According to the varieties, the physico-chemical thermogenesis produced by one gram of turnip seeds put in contact with 1 cc. of water, at 17°C., can reach different maximum rates from 0.5 to 2.5 calories per hour, the most precocious varieties displaying the greatest amount of heat. For wheat grains, in normal conditions of preservation, these rates are around 0.5 calories per hour; for oats, 0.6; for soya beans, 0.25; for spinach seeds, 2; for different varieties of cabbages, 0.4 to 1.5, etc.

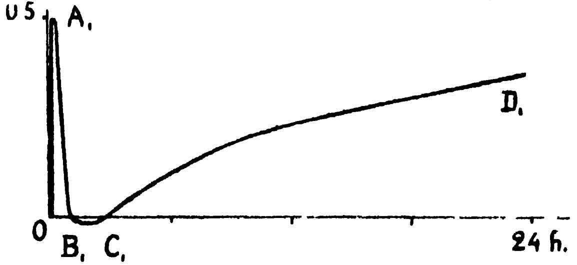


FIGURE 2.—Example of curve for turnip seeds (same letters used as in Fig. 1)

If the seeds are submitted to a previous dehydration, an increase is afterwards observed in their thermogenesis of germination. One gram of wheat grains, previously vacuum-dessicated in presence of phosphoric anhydride during forty-five days, can give, at the moment of hydration, a physico-chemical thermogenesis amounting to the rate of 6 calories per hour (OA_3B, Fig. 3), that is to say twelve times greater than they do in normal conditions. And, what is even more important, their stage of biological thermogenesis itself is magnified (CD_3, Fig. 3). Thus these experiments show the possibility of speeding the germination by a preliminary dehydration of seeds.

Hetero-auxins can also increase biological thermogenesis. On the contrary, if moderate concentration of ethyl alcohol (e.g. 5 per cent) is introduced into the water used for soaking, the biological thermogenesis is delayed. First, the physico-chemical outflow of heat occurs without modification but, after it, the time of latence is indefinitely prolonged. However the seed is not injured, since, being put back into pure water, biological thermogenesis reappears and germination happens normally. On the whole, microcalorimetry is an excellent instrument: (1) to recognize specific physiological differences between seeds, taken in uniform conditions; (2) to appreciate the state of preservation of given lots of seeds, recording their respective aptitudes in regard to germination; (3) to detect their behaviour in presence of various physical and chemical agents In fact, variations supervening on the course of the thermogenesis constitute the most rapid response of the living matter, and the easiest one to be measured accurately, without any perturbing interference of the observer in the course of the phenomenon.

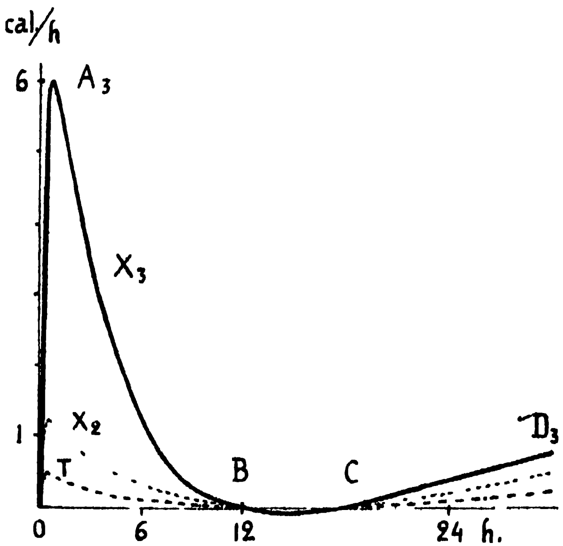


FIGURE 3—Thermogenesis of wheat grains, previously dehydrated during 3 days (dotted curve X_2) and 45 days (solid curve X_3) Dash and dot curve (T) figures the control (same values as in Fig 1)

Thus this method can be valuable in taxonomy as well as for pure physiological purposes. It can be of good use in agronomy to appreciate the reactions and physiological aptitudes of cultivated species, and to develop in this way their genetical study. In another field, we have pointed out its application to the study of bacterial cultures (Calvet, Fricker, and Prat, 1945), showing the diversity of the perspectives offered by this direction of work.

REFERENCES

CALVET, E, FRICKER, J, and PRAT, H (1945). Comptes rendus, Ac. des Sciences, **220** 797.

PRAT, H and CALVET, E. (1944). Comptes rendus, Ac. des Sciences, **218** 944

TIAN, A (1924). Comptes rendus, Ac des Sciences, **178**·705.

TRANSACTIONS OF
THE ROYAL SOCIETY
OF CANADA

SECTION V
BIOLOGICAL SCIENCES

THIRD SERIES—VOLUME XLII—SECTION V

MAY, 1948

Ottawa
The Royal Society of Canada
1948

TABLE OF CONTENTS

Canadian Dietary Patterns: 1937-47. Presidential Address by E. GORDON YOUNG, F.R.S.C. 1

Amelioration of Experimental Hypertension. By JOHN ALLARDYCE, FREEMAN FITCH, and ROBERT SEMPLE 25

Measures of Abundance in Dog-fish (Squalus suckleyi). By W. E. BARRACLOUGH 37

The Sympathicotropic, Interstitial, and Phaeochrome Cells in the Human Foetal Testis. By LOUIS BERGER, F.R.S.C. . . . 45

Serum Alkaline Phosphatase and Alloxan Diabetes in Rats. By MAX M. CANTOR, PERSIS A. WIGHT, and JULES TUBA . . 51

Age and Growth Rate in the Butter Sole, Isopsetta isolepis. By JOHN LAWSON HART, F.R.S.C. 65

Plant Growth in relation to the Bacterial Equilibrium in Soil. By A. G. LOCHHEAD, F.R.S.C. 73

Preliminary Studies on Muskrat Biology in Manitoba. By J. A. MCLEOD 81

Fecundity and Mortality in Pacific Salmon. By FERRIS NEAVE . 97

Giant Axons of Eudistylia vancouveri (*Kinberg*). By J. A. Colin NICOL 107

A Discussion of the Mortality in Pink Salmon (Oncorhynchus gorbuscha) *during Their Period of Marine Life.* By A. L. PRITCHARD 125

The Efficacy of Catch Limitations in Regulating the British Columbia Herring Fishery. By ALBERT L. TESTER 135

Experiments on Human Cutaneous Sensory Thresholds. By I. MACLAREN THOMPSON, F.R.S.C., JAMES G. LUCE, and L. W. DENNY 165

Distribution of Micro-organisms in the Rhizosphere of a Root System. By M. I. TIMONIN and A. G. LOCHHEAD, F.R.S.C. 175

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION V

PRESIDENTIAL ADDRESS

Canadian Dietary Patterns: 1937-47

By E. GORDON YOUNG, F.R.S.C.

MUCH has been said and written of the influence of science on modern warfare, but comparatively little of the influence of war on modern science. Nevertheless one of the most important fibres woven into the fabric of the two world wars of this century has been the feeding of nations.

The analysis of foods has been of great interest to the chemist for over a century. There was available in 1878 the first edition of König's *Chemische Zusammensetzung der menschlichen Nahrungs—und Genussmittel* which in 1903, in the fourth edition, contained 1,500 pages of analytical data. In 1896 Atwater published his analyses of American foods which have served as the foundation for all subsequent determinations of this kind in terms of their content of protein, fat, carbohydrate, and potential energy. Very few systematic analyses, however, have been performed on foods of Canadian origin, and we depend largely on tables compiled in the United States.

The requirements of foodstuffs by animals and their function have been studied scientifically for a much shorter period. This field of knowledge may be considered the science of nutrition. As early as 1824 Brillat-Savarin wrote these prophetic words in his book entitled *La Physiologie du goût:* "La destinée des nations dépend de la manière dont elles se nourissent." The science of nutrition may be said to have been conceived during the latter half of the nineteenth century, in large measure through the imagination and leadership of one man, Carl von Voit (1831-1908), a pupil of Liebig. From Voit's laboratory in Munich there came a guild of investigators who built the ground floor of our knowledge of nutrition in terms of requirements of energy and the relationship of protein, fat and carbohydrate thereto.

In 1906 Hopkins published a short announcement in the *Analyst* to the effect that rats could not live by protein, fat, and carbohydrate alone but required, in addition to mineral salts, traces of some substance which was to be found in milk. This was the first clear conception of what is now recognized as a vitamin. It was soon linked with the etiology of disease by Casimir Funk, in 1913, and he listed such conditions as rickets, scurvy, beri-beri and pellagra as probably caused by dietary deficiencies.

Such was the situation in 1914 at the beginning of the First World War, and by 1917 the supplies of food in Europe were becoming dangerously low. It was not until this time that the nations involved awoke to the fact that the feeding of a nation is a scientific matter to be based on our knowledge of nutrition. What foods in what quantities are necessary for health, for growth, and for life itself? The answer at that time was tragically incomplete and inadequate; the victor was malnutrition in Germany and Austria. The national importance of adequate data, both for a people as a whole and for its various component groups was sketched by Starling in 1919.

From such a stimulus the knowledge of nutrition grew rapidly and for more than twenty years, from 1915 onward, almost monopolized the interests of biochemists. Today it may make some claims to recognition as a science. Credit should be given to the report of Burnet and Aykroyd to the Assembly of the League of Nations in 1935 for drawing the attention of its members to the possibilities of great improvement in public health through better nutrition. The economic depression of 1929-33 had made it abundantly evident that problems of nutrition were not confined to times of war. The Council of the League set up a "mixed committee" on the problems of nutrition which, with the assistance of several bodies such as the International Labour Office, the International Institute of Agriculture, and a technical commission of scientists, submitted a report in four parts to the Assembly in 1936. This report is remarkable for several reasons. In volume II there is set down a scale or dietary "standard" of *average values* which would represent the requirements in terms of calories, protein, and fat of members of a human population subdivided on the basis of age, sex, body weight, and activity. It made a distinction between energy-bearing and protective foods. No quantitative expression of the requirements of minerals or vitamins was given at that time. It emphasized the importance of milk, fresh vegetables, and fruits. It deprecated the use of highly milled cereals and sugar. This report was notable also for compiling statistics of food production and consumption on a world-wide basis (volume IV).

As a result of these presentations and the debate thereon, the seventeenth Assembly of the League recommended to its constituent nations the formation of national committees on nutrition to study national aspects. In response to this formal request the government of Canada set up, in 1938, an advisory committee known as the Canadian Council on Nutrition under the former Department of Pensions and National Health. Since that time this body has given much thought to the food problems of the Canadian people. It is my purpose today to summarize for you what is now known of Canadian dietary patterns, and to attempt to assess the state of nutrition in Canada.

Before so doing, however, I should call your attention to a small volume *Food, Health and Income* by Sir John Boyd Orr, first published in 1936. It analyses the food budgets of 1,152 families collected between 1932 and 1935, in the United Kingdom, subdivided according to income into six classes, representative of the whole population. It shows that the consumption of milk, eggs, meat, fruits and vegetables, rose in amount with increasing income, whereas the consumption of bread and potatoes was practically constant per head of population. It states that the average diet of the poorest group, comprising 4½ million people, was deficient in every dietary essential examined. The second group, comprising 9 million people, was adequate in protein but deficient in all vitamins and minerals considered. Complete adequacy of diet was almost attained in the wealthiest group, comprising only 10 per cent of the population. A brief appraisal of the state of health of the people suggested that statistics of disease and mortality, rate of growth and adult stature, were inversely proportional to level of income. This report created a profound impression and it served a useful purpose by focusing public attention on the relationship of health, nutrition, and agriculture.

At this point it is appropriate to raise the question of the basis on which the adequacy of any diet may be judged. What is the standard of dietary requirements? Orr specifically defines his standard as a diet which will keep people in a state of health or well-being such that no improvement can be effected by a change in dietary intake. This standard may be regarded as the minimum for optimum health within inherited capacity of physical fitness. He adopted the specific dietary requirements of Stiebeling of the United States Bureau of Home Economics (1933) which differ from those of the League of Nations (1936). Neither standard defines "perfect" health or the means of determining it. Since 1936 several such standards have been proposed. The Canadian Council on Nutrition postulated one in 1939.[1] For the sake of uniformity it replaced this standard by the one drawn up by the Food and Nutrition Board of the National Research Council of the United States in 1942. In 1945 these standards were deemed too high in many respects and inadequate in others, and a new statement was prepared and published.[2]

---

[1] *Nat. Health Rev.*, Jan., 1940.

[2] *Canad. J. Pub. Health*, **36**: 272. 1945.

This is now in process of radical revision by the Council, and a new statement will be issued this year. The use of such standards presents certain difficulties which have been analysed by Pett (1945) and Pett, Morrell, and Hanley (1945).

NATIONAL AND PROVINCIAL SURVEYS

In Canada no attempt to assess the nutritional status of the people as a whole or in part had been made when Orr's book was published in 1936. It is only in the past decade therefore that our essential knowledge of Canadian nutrition has been acquired. I became aware of this state of ignorance many years ago when I made a short study of the diets of medical students in 1931. Such information is obtainable by dietary surveys of various kinds. It may be done on the basis of statistics for the country as a whole or for a limited region or social unit of known population. Such statistics of food consumption must be accurate and corrected for several factors such as wastage and volume of home-produced foods before they can yield a true picture. The use of tables of food composition is required and we must assume that any errors involved of the difference between the food eaten and the recorded values are not great. In certain cases these errors compensate one another but in others, as for ascorbic acid, large discrepancies may result (Young and McHenry, 1942). These figures may be expressed per caput or per "man value" as foods consumed, and their constituents as nutrient essentials. Such studies are useful to a government in framing nutritional policies and in following trends in the dietary habits and standards of a country. They fail to show malnutrition in limited groups within a population..

A second type of dietary survey involves measurement by inventory of foods consumed by more or less homogeneous social groups or institutions. Results are calculated in the same way but they can only express the average consumption of the group and fail to indicate the variation within it.

A third type of dietary survey is that applied to the family or individual. It is carried out by direct weighing of the food consumed or by listing purchases and portions served. The former method is the most accurate and informative for the individual but is the most expensive type to use. It is susceptible of errors, however, that are difficult to assess and to overcome. They are psychological in origin and a result of consciousness of the investigation itself—the principle of indeterminacy in nutrition. In general practice the period of observation has been limited to one week but, because of seasonal and practical variations there is some question as to whether this is long enough to give reliable data. Observations of this type may be supplemented by medical examination and various special procedures, such as anthropometric measurements and biochemical analyses of blood and urine, to determine dietary deficiences. Such practice constitutes a nutritional survey. The techniques have been described in a monograph by Bigwood (1939). All types described have been used in Canada during the past ten years and a useful summary was published in 1946 by the Dominion Department of National Health and Welfare under the title *A Report on Nutrition and the Production and Distribution of Food.* Our knowledge of the urban population far exceeds our knowledge of the rural.

The first extensive survey in Canada was carried out during 1937-9 and published by the Dominion Bureau of Statistics (1941). It is a most informative document, and the survey was done at a very opportune time to serve as guide during the six fateful years that followed. The questionnaire method was used to record food purchases during one, two, or three separate weeks by 1,569 families living in twelve cities representing all provinces. The incomes of this group were restricted to the range of $400 to $2,500 per family, and the selection of families was also restricted as to size and living conditions. The percentage of total living expenditure on food varied between 27.6 and 34.8 in the various centres.

TABLE I

ANNUAL FOOD EXPENDITURE IN RELATION TO TOTAL LIVING EXPENDITURE (1938)

	Persons per family	*Per person*	*Percentage of total*
Montreal (British)	4.8	101	34.8
(French)	5.6	84	34.0
Winnipeg (non-British)	4.5	102	33.2
Saint John	4.5	94	33.1
Quebec	4.9	80	32.1
Vancouver	4.4	100	31.0
Winnipeg (British)	4.3	100	30.6
Saskatoon	4.3	100	30.0
Halifax	4.6	95	29.8
Edmonton	4.3	98	29.5
Toronto	4.3	106	29.5
London	4.0	95	28.4
Charlottetown	4.7	83	28.2
Ottawa	4.5	94	27.6

The results showed a wider variation in relation to level of income from 25 per cent in the highest brackets to 40 per cent in the lowest.

These results also varied as the number in the family from 27 per cent with one child to 38 per cent with five children and decreased from $123 per person per year to $72 in the same categories. The distribution of food expenditures amongst the various foods differed comparatively little with income except that the amounts spent on fruits increased with rising income and those on cereal products decreased. Regional similarities were more striking than regional differences. Among French families purchases for meat, white bread, potatoes, canned vegetables, and bananas were high. They were below average for brown bread, cheese, milk, and oranges. In Ontario, purchases of vegetables, bacon, and cheese were notably high, while those for fish were unusually low. Prairie cities were above average for butter, jam, and marmalade. Maritime purchases were relatively high for fish and canned beans but low for veal, dairy products, jam, and marmalade. Seasonal differences were remarkably small. During the war years when rationing became a necessity, these facts were of special significance in their relation to the question of equitable distribution. The records of food purchases were translated into five dietary essentials and expressed per man value per day. The results showed an average consumption of 2,912 Cals., 84.5 gm. protein, 9.5 mg. iron and 0.5 gm. calcium. As compared with the carbon Canadian standards at that time, there was a deficiency of 5 per cent in iron and 13 per cent in calcium but, for the group with the lowest income, deficiencies were shown in all categories, especially in calcium.

TABLE II

FOOD EXPENDITURES AT PROGRESSIVE LEVELS OF FAMILY INCOME FOR ALL FAMILIES

Income	*Expenditure*	
	Per person	*Percentage*
$ 400-$ 799	$ 64	39.3
800- 1,199	86	35.3
1,200- 1,599	100	30.9
1,600- 1,999	108	27.9
2,000- 2,500	116	24.5
Average	98	30.2

Stewart (1936) had carried out a similar survey of the food purchases of families in the same economic range in Edmonton, (pop. 85,774), and Lacombe, (pop. 1,502), Alta., by the questionnaire method. He found a distribution in terms of total food expenditure in Edmonton

TABLE III

DISTRIBUTION OF EXPENDITURE IN FOOD CATEGORIES IN TERMS OF AVERAGES OF ALL FAMILIES IN EDMONTON

Total meat		*Dairy products*		*Cereals*		*Fruits*		*Vegetables*	
Beef	51.2%	Fluid milk	51.0%	Bread	51.4%	Oranges	27.7%	Potatoes	26.5%
Pork	7.6	Cream	5.9	Flour	13.2	Canned fruit	11.7	Tomatoes	20.6
Mutton	3.8	Butter	32.0	Other baking	18.2	Bananas	10.7	Lettuce	15.6
Poultry	3.8	Cheese	4.6	Breakfast foods	12.4	Apples	9.6		
Sausages	3.0	Ice cream	5.5	Other cereals	5.0	Strawberries	9.6		
Bacon	10.7	Canned milk	1.0			Grapefruit	7.5		
Ham	7.6					Lemons	6.4		
Canned	2.5								

as follows: dairy products, 23.6 per cent, meats, 14.6 per cent, cereals, 13.0 per cent, vegetables, 11.5 per cent, fruits, 10.4 per cent, eggs, 4.3 per cent and fish, 2.0 per cent. In Lacombe the proportions were somewhat more for meat, considerably higher for fruit, and relatively less for vegetables. I have calculated from Stewart's data the distribution of purchases within the above categories as percentages, as shown in Table III. Differences between figures for Edmonton and Lacombe are surprisingly small and indicate a characteristic food pattern for the whole area, despite the fact that one is a city and the other a small town.

Stewart's survey also analysed foods purchased in relation to level of income. There was little increase in purchases of sugar, cereals, and eggs on a family basis with rise in income; on the other hand there was a rapid expansion for fruits, fish, and vegetables, especially canned peas and beans, and a slower rise in dairy products and meats.

Sandin, Patrick, and Stewart (1939) extended the Edmonton survey on an inventory basis with twenty-nine families averaging 4.1 members, in receipt of between $500 and $1,500 per annum. The food consumed for one week was measured without regard to wastage, and translated into some of the dietary essentials. These were computed in relation to the Canadian dietary standards. At the lowest level of income, viz., $2 - $5 per week per adult unit, the average consumption was adequate for calories and phosphorus but deficient for protein, calcium, and iron. Approximately half of all the families surveyed showed no deficiencies. Four families were deficient in their consumption of all essentials computed.

Although valuable, such surveys are probably inaccurate and do not contribute essential information on which the nutritional status of the population may be judged. In 1939, McHenry published a statement of a dietary survey in Toronto by the method of individual weighings. It included 602 individuals in 100 families in the low-income class receiving an average of $1,020 per annum. The average number of children per family was 4.2. The dietary records were translated into calories, protein, calcium, and iron. The average caloric intake for the men was 2,360 and for the women, 1,720, or 77 and 70 per cent respectively of the standard set up by the Canadian Council on Nutrition in 1939. Men secured 86 per cent of the protein recommended, women 73, and children 75. While adults seemed to ingest sufficient calcium, the children were markedly deficient at only 57 per cent, showing an insufficient consumption of milk and cheese. The diets of the women and children were deficient in iron. An interesting fact was established by this survey: that the father was the best-fed member of the family and the mother was the worst. The survey pointed to possible dietary deficiencies hitherto unsuspected in Canada.

The Canadian Council on Nutrition then undertook to investigate more fully the situation and surveys in four urban centres, Halifax, Quebec, Toronto, and Edmonton by the method of individual weights. Each consisted of observations over one week during 1939-40 (Young, 1941; Sylvestre and Nadeau, 1941; Patterson and McHenry, 1941; Hunter and Pett, 1941). The limits of annual income were between $500 and $1,500 in Halifax, Quebec, and Edmonton, and $1,500 to $2,400 in Toronto. In this group the records of wastage were found to be so small as to be negligible. Expenditures on food were at a level of about 40 per cent of income. The results are compared in Table IV with the Canadian standards at the 70 per cent level. The first survey in Toronto (McHenry, 1939) is also included in Table IV. McHenry's conclusion (1939) of the inequitable distribution of food within the family was confirmed. The table shows important deficiencies in minerals and vitamins, especially calcium, thiamine, and ascorbic acid. This points to inadequate consumption of whole milk, citrus fruits, and the leafy, green, and yellow vegetables. The first survey in Toronto was carried out about a year previous to the other four. Because of the limited character of these surveys, there is no way of calculating from them the nation's dietary status. The conclusion of deficiency cannot be sound until supported by physiological data and by clinical and other examinations. On the other hand the results do show dietary patterns.

TABLE IV

COMPARISON OF DIETS IN FOUR CITIES ON THE BASIS OF A 70% LEVEL OF THE CANADIAN DIETARY STANDARD (1939) AS PERCENTAGE DEFICIENT

City	*Families*	*Size*	*Calories*	*Protein*	*Calcium*	*Iron*	*Vitamins*		
							A	*B_1*	*C*
Halifax	82	4.7	4	4	40	10	34	100	57
Quebec	76	5.1	1	1	46	8	63	60	13
Toronto (500-1500)	100	6.2	33	37	60	75	43	93	79
Toronto (1500-2400)	80	3.9	4	3	3	6	6	57	31
Edmonton*	76	4.3	9	12	27	27	23	77	23

*On basis of 60 per cent of Canadian Dietary Standards.

From the Halifax data I have worked out this aspect in greater detail (Young, 1942) within the group in relation to income level. The data show that the consumption of dairy products, meat, and fish was reduced most markedly with falling expenditures. The consumption of bread and potatoes was relatively constant at all levels. Forty per cent of families were using small amounts of whole-wheat bread and 66 per cent were buying patented breakfast cereals. Not all families were consuming fresh milk, and 34 per cent depended in whole or in part on evaporated or condensed varieties. Very few housewives made their own bread, in contrast to a rural group in Nova Scotia (McNally *et al.*, 1943). The results are shown in Table V in comparison with averages for similar groups. The high consumption of fish and apples reflects the close relationship that may exist between the diet of the people and their environment. The low consumption of milk and cheese is another notable feature of these diets.

TABLE V

COMPARISON OF CONSUMPTION OF COMMON FOODS
(per person per week)

	Halifax	*Canada*[1]	*Great Britain*[2]	*North Atlantic States (U.S.A.)*[3]
Income ($)	1.08-13.94	1.92-11.83	1.25-5.75 +	2.96-15.51
White bread (lb.)	2.04	2.62	3.65	1.87
Flour (lb.)	0.83	0.63	–	0.54
Butter and margarine (lb.)	0.62	0.61	0.65	0.40
Eggs (oz.)	8.4	6.44	- -	8.9
Cheese (oz.)	0.9	1.28		2.37
Milk (qt.)	1.67	2.28	2.34	2.35
Fish (lb.)	0.74	0.18	0.49	0.40
Meat (lb.)	2.21	1.60	2.04*	2.27
Potatoes (lb.)	3.12	3.20	–	3.02
Sugar (lb.)	0.80	0.91	1.74	1.08
Citrus fruits (lb.)	0.39	0.42		0.83
Apples (lb.)	0.83	— -		0.67

[1] Dominion Bureau of Statistics, *Family income and expenditure in Canada, 1937-38.*

[2] Cathcart, E. P., and Murray, A. M. T., *Medical Research Council special report,* no. 218, 1937. (Expressed per man value.)

[3] Stiebling, H. K., and Phipard, E. F., U.S. Dept. of Agriculture, Circular no. 507, 1939.

In comparison with the Halifax results we have the observations of McNally, Eaton and MacNeil (1943) in the Annapolis Valley, N.S., comprising records for one week in 1942 taken by average servings. There were sixty-one families of prosperous farmers with an average constitution of 5.4 members per family. The most marked deficiencies were in calcium, thiamine, riboflavin, and ascorbic acid—identical with the findings in Halifax. The degree of deficiency however was not as severe; thus only 4 per cent were below the 70 per cent level of the Canadian standard for vitamin A, 30 per cent for vitamin B_1, 36 per cent for vitamin C, and 20 per cent for calcium. A high proportion of families grew their own fruits and vegetables and most bread was home-made, usually from white flour. Few made their own butter.

During 1945, and the winter and spring of 1946, the Division of Nutrition of the Department of National Health and Welfare conducted an extensive survey of rural areas in several counties in the three Maritime Provinces. Food consumed by families over three separate weeks was entered on check-lists. The results for these provinces have just been

TABLE VI

COMPARISON ON THE BASIS OF PROVINCES

Amounts of Foods in Pounds consumed per Person per Week in Rural Families in the Maritime Provinces, 1945-6

Food	*Canada*[1]	*N.S.*[3]	*N.B.*[3]	*P.E.I.*[3]
Milk—whole	8.19	9.55	7.38	8.64
Milk—solids (lb.)	1.30	1.66	1.33	1.61
Cheese	0.09	0.13	0.11	0.15
Meat	2.69	1.84	1.57	1.96
Beef	1.16	1.21	0.88	1.32
Pork	1.06	0.49	0.60	0.53
Poultry	0.52	0.27	0.25	0.39
Fish	0.18[2]	0.60	0.46	0.47
Eggs	0.75	0.79	0.64	0.87
Butter	0.55	0.50	0.56	0.55
Sugar and syrups	1.52	1.06	1.11	1.06
Potatoes	3.65	5.58	6.37	5.74
Citrus fruits and tomatoes	1.83	1.72	1.28	1.32
Other fruit.	1.17	3.07	2.79	3.30
Vegetables				
leafy green and yellow	0.99	2.83	1.82	1.83
other	0.99	2.19	1.46	2.19
Grain products	3.85	4.62	5.13	5.04

[1] *Food consumption levels in the United States, Canada and the United Kingdom,* 1946.

[2] Dominion Bureau of Statistics, *Family income and expenditure in Canada, 1937-1938.*

[3] Dominion Department of National Health and Welfare, unpublished data.

collated (Pett, 1948) and are shown in Table VI together with the Canadian average. While the differences are small, they show certain definite patterns. Most figures for New Brunswick are lower than these for either Nova Scotia or Prince Edward Island with the exception of pork, sugars, potatoes, and bread, which are higher. Meat, with respect to poultry and pork, is at a lower level than the all-Canada average. This is also true of sugar and syrups, and citrus fruits. The consumption of fish, potatoes, other fruits, green vegetables, and grain products, is higher in the Maritimes. Even within counties the effects of local environment are evident in the consumption of fish, fruit, potatoes, or dairy products.

In December, 1942, the federal Division of Nutrition (Pett, 1943a) carried out by the questionnaire method an extensive survey across Canada covering 1,384 families and about 5,000 individuals. It was based on the degree of conformity to Canada's Food Rules as a guide to adequate nutrition. While it showed marked deviations from and non-conformity to these rules, this type of survey could not assess whether the dietary essentials were adequately represented in the diets taken. However, much information on food habits was tabulated. The major findings were as follows:

Milk: 11 per cent did not drink milk; 54 per cent had less than the recommended minimum.
Cheese: 24 per cent had no cheese.
Fruits: 54 per cent had no citrus fruit or tomato daily.
Vegetables: 66 per cent did not have a green or yellow vegetable daily.
Cereals: 72 per cent had less than four slices of bread daily; 91 per cent did not have a whole-grain cereal daily.
Eggs: 43 per cent had fewer than four eggs weekly.

Hopper has directed numerous studies on the consumption of particular foods, especially milk, for the Dominion Department of Agriculture (Hopper and Boucher, 1938). The data have been reviewed relative to income (Hopper, 1943) in both rural and urban areas. Consumption of milk per caput was 0.74 pint per day of which 62 per cent (0.46 pint) was used as a beverage. Daily intake was definitely higher on the farm than in the city, and was found to be directly proportional to income. Persons of British origin were amongst the largest users, and French-speaking Canadians were slightly below the average throughout Canada. A large proportion of adults did not drink milk, and at the highest income level 68 per cent were in this class. Among users the average daily consumption was 1.27 pints. In families on relief the proportion of children not drinking milk was 33 per cent. Many teen-age children in cities did not drink milk. Education within the mess-halls of the armed services during the war should have done much to improve this situation.

SURVEYS ON CANADIAN CHILDREN

From the School of Hygiene of the University of Toronto, under the leadership of McHenry, has come a series of significant nutritional investigations of adolescent school-children in East York Township of Ontario. They were based on a broad nutritional survey including dietary studies, medical and dental examinations, and certain special diagnostic tests. The children ranged in age from eleven to nineteen years—260 boys and 286 girls. Dietary records were kept on the basis of servings. The standards for comparison were the dietary allowances recommended in the United States, which were identical at that time with Canadian standards. Riggs *et al.* (1943) reported that the most striking finding was the low consumption of foods containing thiamine and ascorbic acid. Consumption of foods containing calcium and riboflavin was fair, and of other dietary essentials reasonably high. Fifteen per cent of the children were receiving vitamins A and D in the form of fish liver oil. The state of health, physical and nutritional, was assessed and an attempt was made to correlate it with the dietary record. Broadly stated, this attempt was unsatisfactory, with many marked discrepancies. The results of this survey raised several important possibilities: (1) the standards adopted were too high; (2) a dietary record of one week was too short to be representative; (3) methods of assessing deficiencies were inadequate.

From the results of the same study Ferguson, Leeson, and McHenry (1944) recalculated observed values for consumption of thiamine and ascorbic acid on the basis of different standards proposed, with thiamine at 0.23 mg. per 1,000 Cals. and ascorbic acid at 30 mg. per day. The picture of inadequacy was thus largely revised and fitted the physical findings much better. The standard figures for these nutrients would appear to be too high on the basis of this evidence. As reconsidered, 71 per cent of the girls and 72 per cent of the boys had excellent or good dietary histories. Ferguson and McHenry (1944) repeated the dietary survey on only 75 children of the original group still in school after an interval of two years. The results were qualitatively the same as in the first survey but quantitatively somewhat improved, especially in terms of ascorbic acid. More bread, milk, and citrus fruit were consumed, especially by the boys. Economic conditions had improved and a federal subsidy had come into operation with citrus fruits. The average intakes of girls and boys in the two surveys are given in Table VII. Rationing had shown no apparent effect on the ingestion of dietary essentials in January of 1944.

In another paper Virtue and McHenry (1945) compared the results of observed energy values of the diets in the East York survey with the

TABLE VII

AVERAGE INTAKES OF DIETARY ESSENTIALS IN EAST YORK SURVEY

Nutrient	*Girls*		*Boys*	
	1942	1944	1942	1944
Calories	2,454	2,540	3,032	3,599
Protein (gm.)	81	80	95	110
Calcium (gm.)	1.1	1.2	1.4	1.7
Iron (mg.). .	14	14	18	20
Vitamin A (I.U.)	7,870	6,070	10,820	10,580
Thiamine (mg.) .	0.9	1.0	1.1	1.4
Riboflavin (mg.)	2.0	2.1	2.3	2.9
Ascorbic acid (mg.). . .	46	56	52	68

recommended allowances. They found that the Toronto children, in terms of the mean, were receiving diets slightly lower than the standards.

A broad nutritional survey was accomplished by Pett and Hanley (1947) with a team of workers during January and February of 1946 which involved 1,495 pupils in sixteen schools spread over five areas in British Columbia. Most of the children were five to eleven years of age and the sample constituted 1.74 per cent of this age group within the province. During May and June of 1946 the survey was extended to include 1,465 pupils in forty-three schools in Saskatchewan, constituting 0.85 per cent of children of this age category in the province. Dietary records were obtained on a simplified questionnaire based on number of servings with especial reference to Canada's Food Rules. Physical examinations were made by medical personnel with special experience in the field of nutritional deficiencies. Levels of some vitamins were determined, as well as haemoglobin and serum proteins, in blood. The results are summarized in Table VIII. This is a very informative table.

TABLE VIII

NUTRITIONAL SURVEY OF SCHOOL-CHILDREN (5-11 YEARS) BASED ON ONE OR MORE SIGNS OF DEFICIENCY IN PERCENTAGE OF PROVINCIAL GROUP

Deficiency	*British Columbia*		*Saskatchewan*	
	1 sign only	Total	1 sign only	Total
Vitamin A	19.9	21.6	19.9	21.4
Riboflavin	42.1	81.5	39.6	53.3
Niacin	2.3	2.3	2.3	2.3
Ascorbic acid.	3.8	3.9	12.9	13.9
Rickets (healed)	40.3	56.8	41.4	51.0

Few diseases, clinically considered, have a single sign which is completely pathognomonic. The finding of two or more greatly increases the probability of the potentiality of that disease. Furthermore, signs of mild nutritional deficiencies are not specific. Poor posture and carious teeth were noted in moderate degree in approximately 50 per cent of all children and in marked degree to the extent of 10 per cent in British Columbia and 3 per cent in Saskatchewan. Little or no reserve supplies of ascorbic acid were inferred from levels estimated in blood in 40 per cent of children in British Columbia and 56 per cent in Saskatchewan with less than 0.4 mgm. per cent as the line of demarcation. Concentrations of serum proteins were below 6 per cent in 18 per cent of the children in British Columbia and 4 per cent of the children in Saskatchewan. No deficiency disease in acute form was found in this survey. Dietary inadequacies however were encountered frequently. Underweight and evidence of healed rickets were the most important findings from physical examination. Riboflavin deficiency in the diet was widespread, yet ariboflavinosis was not noted. This survey again suggests as concluded previously from the East York survey, that the standard of comparison has been set too high.

Miss Ada MacLeod and I have just completed a nutritional survey of fifty-five children in Halifax, N.S., and a compilation of the diets of ninety-three children in the four surveys of 1939 (Young and MacLeod, 1948) in Halifax, Quebec, Toronto, and Edmonton. It was designed to study children of pre-school age between one and five years of age. The method of individual weighings was used, and two dietary records for one week were obtained six months apart. Physical, anthropometric, and laboratory examinations were made, and age of calcification was determined by radiographs in some cases. The survey was designed to obtain factual information about diets in terms of the levels of essential nutrients on which children could and did grow normally. Our findings are difficult to present simply without an overdose of statistics. They may be briefly summarized by stating that (1) energy and protein values of the diet have been more closely related to age in graphic form, (2) the present practice in North America whereby fat constitutes about 35 per cent of the energy value of the diet was confirmed, (3) the findings suggest that consumption of milk and fish liver oil diminishes too early in child development, between three and four years of age as shown by the decreased ingestion of calcium and vitamin D. Our results tend to show that the period of transition from an essentially milk diet to one of mixed foods is a critical one, a conclusion supported by the sharp drop in levels of intake of most dietary essentials measured on the basis of intake per kilogram of body weight or per 1,000 Calories.

A survey of school-children in Gaspésie revealed numerous cases of anaemia and some evidence of avitaminosis in relation to the B-complex and ascorbic acid in about 70 per cent of children examined (Ball *et al.*, 1946).

SURVEYS OF MISCELLANEOUS GROUPS

Apart from the surveys in Edmonton previously referred to, few dietary observations have been made in central or western Canada. Hiltz (1943) reported a study of twenty-nine families in Winnipeg comprising 165 persons within the income group of $948-$1,764 per year. This survey was carried out on the inventory method. The results confirmed the earlier observations noted in the four Canadian surveys in Halifax, Quebec, Toronto, and Edmonton with respect to thiamine and ascorbic acid. As shown by the results of the East York survey, the level of intake of the standard is open to question.

Waagen and Pett (1942) investigated the diets of a group of male students at the University of Alberta over a period of one year in relation to ascorbic acid. They found that from November to May the average consumption was 41 mg. and from June to October it was 82 mg. Although they obtained evidence from levels of urinary excretion and by administration of ascorbic acid that the tissues of such individuals were not saturated in the winter, no signs of deficiency were observed. They pointed out, however, the importance of citrus fruits in the diets as essential contributors to the adequate intake of vitamin C.

Moore, Kruse, Tisdall, and Corrigan (1946) have reported a nutritional survey amongst the Cree Indians of northern Manitoba. There are approximately 126,000 Indians in Canada constituting about 1 per cent of our population. Most of them dwell in the hinterland of northern Canada. This study was concerned with a typical group of 400 "Bush" Indians whose yearly income was approximately $400. Sanitary conditions were primitive and epidemics were frequent. The infant mortality was very high reaching a figure of 400 per 1,000 live births. The foods most commonly purchased were white flour, lard, sugar, tea, and a small amount of oatmeal. These were supplemented by berries, game, fish, and potatoes. Dietary consumption for the group was estimated on the basis of store purchases, and the result showed notably low levels of most essentials. No estimate of additional food could be made under the circumstances, but the authors state that it must have been small. However no single deficiency disease was encountered in physical examinations, although frequent abnormalities of conjunctivae, ocular limbic blood vessels, tongue, and gums were observed. Lachrymation, photophobia, and diminished acuity of vision were general complaints. The rôle of nutrition in this picture remains to be established by therapeutic trials as distinct from climatic conditions.

Rabinowitch (1936) made a few observations of the diet of Eskimos and of their general physical condition in the Canadian Eastern Arctic in 1935. This area has a population of about 2,400 and in the regions visited lived about 950 natives or 40 per cent of the total. Of these 389 Eskimos were examined. White flour was very common where hunting was poor. Meat in the form of seal, walrus, whale, and game birds was common. Caribou, and dried buffalo meat were also relied upon by the natives. Polycythaemia, with haemoglobin at 145 per cent, was frequently found. No evidence of rickets was observed, but severe conjunctivitis, pterygia, snow-blindness, and blepharitis were frequent, suggesting probable avitaminosis. Rabinowitch made a rough estimate that the average diet of the adult would contain 30-40 gm. of carbohydrate, 250-300 gm. of protein, and about 150 gm. of fat.

Several studies have been made by Tisdall and his co-workers on restricted groups in Toronto. In 1944, Ebbs, Tisdall, and Scott published the results of a dietary investigation of about 1,000 school-teachers on the basis of dietary records for one week during 1941 and 1942, repeated twice. They also tested the effect of supplementary vitamins on general health in one sub-group. The nutritional levels shown by the averages of dietary essentials were sufficiently high that no marked improvement in state of health measured superficially could be shown either by dietary improvement or supplement of vitamins.

In important studies carried out on 400 expectant mothers in Toronto in 1941, Ebbs *et al.* (1941; 1942a) determined the effects of improved nutrition in relation to infant mortality. Infants and expectant mothers have been recognized for some time as particularly vulnerable to dietary deficiencies. A dietary record was obtained for one week from volunteers of those attending the prenatal clinic of the Toronto General Hospital. This group was sub-divided so that 120 were allowed to continue on their ordinary, relatively poor diet and 90 were given supplementary foods. An additional 170 were advised as to how their diets could be improved. The obstetrical histories of these women were followed and it was found that a much higher incidence of miscarriage, stillbirths, premature births, and minor complications occurred in those women on an uncontrolled, poor diet. The weight of the baby at birth, however, was not affected.

In another correlated study, Ebbs *et al.* (1942b) observed the influence of improved prenatal nutrition of the mother upon the subsequent mortality and morbidity of the infants during the first six months of life. They conclude that the statistics of these conditions were definitely improved. The number of women and children in this investigation was not large and the results would be more convincing if this important study could be extended.

NUTRITION IN CANADA DURING WORLD WAR II

We now come to a consideration of aspects of nutrition in Canada in World War II. One part of the story is told in two publications of the government of Canada, *Canadian Food and Nutrition Statistics (1946)* and *Food Consumption Levels in Canada, the United Kingdom and the United States: Reports of 1944, 1945 and 1946*. The statistics represent the best available estimates of foods moving into civilian consumption and are expressed as amounts distributed in Canada at the level of the retail store. No corrections are applied for losses by spoilage, handling, or cooking below the retail level. The extent of these losses may be judged by reference to Table IX. They are quite considerable and vary depending on culinary practices especially for vitamins B_1, B_2, C, and niacin. Numerous other corrections have been applied, however. The values have been translated into quantities of essential nutrients as mean values per head of population per day. Of foods contributing in greatest measure to the nutriture of Canadians, dairy products, meats, and cereals are outstanding and ahead of other articles in the diet. Vegetables are next in importance, and fruits in fifth place. The results are shown in Tables X to XII.

The energy value of the average diet rose steadily during war years but declined in 1945 due to sharp declines in supplies of meat, potatoes, and sugar. Despite apparent shortages in meat and fats, the average consumption was greater than that of the pre-war years. Calcium consumption reflects the great increase in the production of whole milk. The increase, in 1942, of vitamin A was the result of a doubled consumption of carrots and increases in greens and butter. The level of ascorbic acid was well maintained through the war and rose after the war due to several economic factors. We may thus conclude that the period of the war was one of better nutrition for the Canadian people owing to improvement in economic conditions, more equitable distribution due to rationing, and increased production of foodstuffs. These figures, however, are averages and must not be taken to indicate that no malnutrition can exist in Canada. Rationing in Canada may be said to have had a beneficial effect where, by control of prices and distribution, meat and milk consumption in the lower income groups was increased, although it may have been reduced by as much as 35 per cent in the higher income group.[3] The worst that may be said of rationing in Canada is that it

---

[3] *Consumer Facts*, no. 9, 1943.

TABLE IX

ESTIMATED EDIBLE WASTAGE AND LOSSES BY COOKING IN THE HOME*

Foods	*Loss*	*Nutrients*	*Loss*
Dairy products	1.9%	Calcium	2%
Meats and fish	7.6	Iron	6
Eggs	1.0	Thiamine	30
Fats	5.1	Riboflavin	15
Sugars	2.0	Niacin	20
Potatoes	4.9	Ascorbic Acid	35
Tomatoes and citrus fruits	8.2		
Other fruits and vegetables	3.5		
Green and yellow vegetables	7.0		
Grains	7.6		

**Report on nutrition and the production and distribution of Food*, 1946, pp. 153-4.

caused some dislocation of food habits, particularly in the higher income class. It is worthy of note that the rates of infant and maternal mortality decreased between 1939 and 1945.

It should be noted briefly that the export of food during the period of the war as compared with the pre-war period of 1935-9 has been remarkably high for wheat, concentrated milk, butter and cheese, beef, pork, bacon and canned meat, and eggs. Thus, in 1944, the percentage

TABLE X

ESTIMATED SUPPLIES OF FOODS USED BY CANADIAN CIVILIANS

(average per person per year in pounds)

Food groups	1935-9	1940	1941	1942	1943	1944	1945
Milk solids	56	57	58	61	65	67	68
Tomatoes and citrus fruits	58	68	77	83	77	109	95
Vegetables							
Potatoes	193	191	201	199	211	200	193
Leafy, green and yellow	44	42	44	62	40	47	52
Other	34	31	27	41	26	56	52
Cereals	208	175	180	195	221	198	200
Meats	118	122	130	139	156	149	140
Poultry, game and fish	35	34	34	40	42	42	40
Eggs	31	30	30	32	35	36	39
Fats	41	41	45	47	42	41	37
Sugars and syrups	104	108	111	91	88	98	79

TABLE XI

ESTIMATED SUPPLIES OF NUTRIENTS IN FOODS USED BY CANADIAN CIVILIANS
(average per person per day)

Nutrient	1935-39	1940	1941	1942	1943	1944	1945
Calories	3,064	2,966	3,100	3,138	3,265	3,235	3,055
Protein (gm.)	91	86	89	96	103	100	99
Fat (gm.)	116	118	124	130	131	131	123
Carbohydrate (gm.)	413	390	407	396	418	414	388
Calcium (mg.)	829	856	873	925	967	1010	1003
Iron (mg.)	13	12	13	14	14	15	14
Vitamin A (I.U.)	6,682	6,590	6,450	8,210	6,520	7,150	7,300
Thiamine (mg.)	1.46	1.42	1.47	1.61	1.72	1.74	1.66
Riboflavin (mg.)	1.77	1.78	1.82	1.94	2.04	2.09	2.06
Niacin (mg.)	16.2	15.3	16.3	16.9	17.7	18.4	17.6
Ascorbic Acid (mg.)	77	76	82	87	86	99	97

TABLE XII

CONTRIBUTION OF EACH FOOD TO TOTAL NUTRIENTS IN 1943
(as percentages of totals)

	Calories	*Protein*	*Fat*	*Carbohydrate*	*Calcium*	*Phosphorus*	*Iron*	*Vitamin A*	*Thiamine*	*Riboflavin*	*Niacin*	*Ascorbic acid*
Dairy products*	13	23	18	7	78	36	8	21	9	51	3	17
Fruits												
Tomatoes and citrus	s	s	s	1	1	s	1	3	2	s	1	18
Other	2	s	s	3	s	s	3	2	1	1	2	6
Vegetables												
Potatoes	6	5	s	10	2	6	10	2	9	5	13	35
Green and yellow	s	s	s	s	2	1	3	48	2	1	3	13
Legumes	2	3	s	2	2	4	7	0	5	2	3	0
Cereals...	30	31	2	50	6	22	26	0	21	7	15	0
Meats ..	14	23	30	s	2	16	25	9	44	20	50	3
Poultry and fish	2	7	3	s	s	5	4	s	2	2	10	1
Eggs	2	6	4	s	2	5	7	6	4	7	s	0
Fats .	15	s	41	s	s	s	s	8	s	s	s	0
Sugars and syrups	12	0	0	24	2	s	3	0	s	s	0	0

s = small, less than 1 per cent

*Dairy products do not include butter

increase in export of wheat was 79; of beef, 2,564; of bacon, 217; of eggs, 4,483.[4]

Public interest has been aroused in this newer knowledge of nutrition, and the results of the research laboratory are apt to pass too quickly to application in everyday life without adequate and critical confirmation. There are few subjects in science that come so close to the life and interest of the general public as does food. Few branches of science have such possibilities as this elevation of the general level of the health of mankind with its many subsidiary benefits. There is thus the unusual danger of the scientist losing his objective outlook and becoming a protagonist. Yet the great value of science lies in its service to man. There must therefore be a dilemma and a compromise. The application of nutrition to problems of the feeding of nations has been greatly increased by the formation of the Food and Agricultural Organization of the United Nations and the first World Food Survey (1946). The report includes seventy countries and 90 per cent of the earth's population. It states that about half of the world's population subsisted before the war at a level of food consumption which was not high enough to maintain normal health, growth, and normal work. Such a statement is based on the energy value of 2,250 Calories per caput, and the conclusion is lacking in adequate scientific evidence. From this World Food Survey, however, it is established that Canada is among the few best-fed nations of the world. I have reviewed the evidence for this and also for the presence of some malnutrition. At the age of ten, in 1939, boys were 1.5 inches taller and 3.8 pounds heavier than in 1923. About 70 to 80 deaths occur in Canada yearly due to deficiency diseases, mostly rickets (Pett, 1944). The comparative absence of deficiency states, however, does not mean that higher levels of food intake would not have beneficial effects on health. It has been estimated that it would be necessary to increase Canadian production of fluid milk by about 25 per cent over the 1944 level, green vegetables by 26 per cent, and tomatoes by 50 per cent, to provide adequate quantities for home consumption and the maintenance of present levels of export (*Report on Nutrition,* 1946; Pett, 1943b). Such a goal is well within the immediate capacity of Canadian Agriculture.

During the present century there have been notable changes in food habits in Canada. The consumption of refined sugar has risen, and so also has that of citrus fruits, green vegetables, and milk. The factors are mainly economic and educational. It is a challenge to governments and statesmen so to shape our national food policy that freedom from want leading to optimum health may be achieved.

---

[4] *Consumer Facts,* no. 27, 1945.

REFERENCES

Atwater, W. O. and Woods, C. W. (1896). U.S. Dept. Agr., Bull. no. 28.

Ball, M. V., Brouha, L., Gasnier, A. and Tremblay, J. L. (1946). Laval Med., **11**: 1025.

Bigwood, E. J. (1939). Guiding principles for studies on the nutrition of populations. Geneva, League of Nations, Health Organization.

Burnet, E. and Aykroyd, W. R. (1935). League of Nations, Quart. Bull. of the Health Organization, **4**, no. 2.

Canadian food and nutrition statistics, 1935 to 1945 (1946). Ottawa, King's Printer.

Construction and use of dietary standards (1945). Canad. J. Pub. Health, **36**: 272.

Dominion Bureau of Statistics (1941). Family income and expenditure in Canada, 1937-38. Ottawa, King's Printer.

Ebbs, J. H., Brown, A., Tisdall, F. F., Moyle, W. J., and Bell, M. (1942b). Canad. Med. Assoc. J., **46**: 6.

Ebbs, J. H., Scott, W. A., Tisdall, F. F., Moyle, W. J., and Bell, M. (1942a). Canad. Med. Assoc. J., **46**: 1.

Ebbs, J. H., Tisdall, F. F., and Scott, W. A. (1941). J. Nutrition, **22**: 515.

———(1944). Canad. Med. Assoc. J., **50**: 208.

Ferguson, H. P., Leeson, H. J., and McHenry, E. W. (1944). Canad. J. Pub. Health, **35**: 66.

Ferguson, H. P. and McHenry, E. W. (1944). Canad. J. Pub. Health, **35**: 241.

Food consumption levels in the United States, Canada and the United Kingdom (1944-6). Ottawa, King's Printer.

Hiltz, M. C. (1943). Canad. J. Pub. Health, **34**: 6.

Hopper, W. C. (1943). Canad. J. Econ. and Pol. Sci., **9**: 487.

Hopper, W. C. and Boucher, G. P. (1938). Dominion Dept. Agr., Pub. no. 608.

Hunter, G. and Pett, L. B. (1941). Canad. Pub. Health J., **32**: 29.

König, J. (1903). Chemische Zusammensetzung der menschlichen Nahrungs—und Genussmittel, 4th ed. Berlin, Springer.

League of Nations (1936). Pub. II Economic and Financial II B, 3, 4, 5 and 6.

McHenry, E. W. (1939). Canad. Pub. Health J., **30**: 4, 431.

McNally, F. M., Eaton, E., and MacNeill, A. (1943). A dietary survey in rural Nova Scotia. Wolfville, N.S., Acadia University.

Moore, P. E., Kruse, H. D., Tisdall, F. F., and Corrigan, R.S.C. (1946). Canad. Med. Assoc. J., **54**: 223.

Orr, J. B. (1936). Food, health and income. London, Macmillan.

Patterson, J. M. and McHenry, E. W. (1941). Canad. Pub. Health J., **32**: 21.

Pett, L. B. (1943a). Recent dietary surveys in Canada. Ottawa.

———(1943b). Canad. J. Pub. Health, **34**: 1.

———(1944). Canad. Med. Assoc. J., **50**: 9.

———(1945). Canad. J. Pub. Health, **36**: 69.

———(1948). Unpublished data.

Pett, L. B. and Hanley, F. W. (1947). Canad. Med. Assoc. J., **56**: 187.

Pett, L. B., Morrell, C. A., and Hanley, F. W. (1945). Canad. J. Pub. Health, **36**: 232.

Rabinowitch, I. M. (1936). Canad. Med. Assoc. J., **34**: 487.

Recommended dietary allowances (1945). U.S. National Research Council, Reprint and Circular Series, no. 122.

Report on nutrition and the production and distribution of food. (1946). Ottawa, King's Printer.

Riggs, E., Perry, H., Patterson, J. M., Leeson, J., Mosley, W., and McHenry, E. W. (1943). Canad. J. Pub. Health, **34**: 193.

Sandin, M., Patrick, M., and Stewart, A. (1939). Canad. J. Pub. Health, **30**: 177.

Starling, E. H. (1919). The feeding of nations. London, Longmans Green.

Stewart, E. H. (1936). Consumers' expenditures on food commodities, Parts I and II. Edmonton, University of Alberta.

Stiebeling, H. K. (1933). U.S. Dept. Agr., Misc. Pub., no. 183.

Sylvestre, J. E. and Nadeau, H. (1941). Canad. Pub. Health J., **32**: 11.

Virtue, R. and McHenry, E. W. (1945). Canad. J. Pub. Health, **36**: 240

Waagen, H. K. and Pett, L. B. (1942). Canad. J. Research, **B 20**: 246.

World food survey (1946). Washington, F.A.O. Publication. July 5.

Young, C. M. and McHenry, E. W. (1942). Canad. J. Pub. Health, **33**: 224.

Young, E. G. (1931). Canad. Med. Assoc. J., **24**: 440.

———(1941). Canad. Pub. Health J., **32**: 6.

———(1942). Canad. Pub. Health J., **33**: 480.

Young, E. G. and MacLeod, A. V. (1948). Unpublished data.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Amelioration of Experimental Hypertension

By JOHN ALLARDYCE, FREEMAN FITCH, and ROBERT SEMPLE

Presented by A. H. HUTCHINSON, F.R.S.C.

THE problem of hypertension has been attracting considerable attention, particularly in the last ten years. Four main experimental approaches to the problem have been followed by the various workers: (*a*) Renal origin—Goldblatt, Lynch, Hanzal, and Sommerville (1934) induced hypertension by unilateral nephrectomy together with partial occlusion of the renal artery to the remaining kidney; Page (1939) and Abramms and Sobin (1947) by encapsulating the kidneys. (*b*) Nervous origin—Schafer (1944) and Thomas (1947) have induced hypertension by sectioning of the aortic depressor and carotid sinus nerves. (*c*) Vascular origin—King (1947) has shown a definite thinning out of the elastomeric constituents of the aortic wall and an accumulation of collagenous fibres and other deposits with age. (*d*) Hormonal origin—Knowlton, Leob, Stoerk, and Seegal (1947) have confirmed previous findings that overdosage of rats with desoxycorticosterone acetate (DCA) results in renal and cardiac hypertrophy, changes in the adrenal cortex, and hypertension, while Grollman (1946) has reduced or abolished these effects by drastically reducing the sodium chloride intake.

After careful consideration of the above approaches, it was decided to investigate further the role of hormone disbalance as the source of hypertension. From the well-known vasodilatory action of histamine and the success attained by Wirtschafter and Widman (1947) in ameliorating peripheral vascular disease by injection of histamine and sodium ascorbate, it seemed reasonable that histidine and ascorbic acid might prove satisfactory in ameliorating hypertension induced by DCA, or in preventing its occurrence.

METHODS

Wistar albino rats were used as the experimental animals. Systolic blood pressures (BP) were determined by the indirect method using the foot. The rats were lightly anaesthetized by intra-peritoneal injection of just enough sodium pentothal to hold them under for thirty to sixty minutes. The apparatus used for measuring the BP was a modification of that used by Griffith and Farris (1942) and is shown in Fig. 1. A strong light was directed through the thin tissue between the toes, which

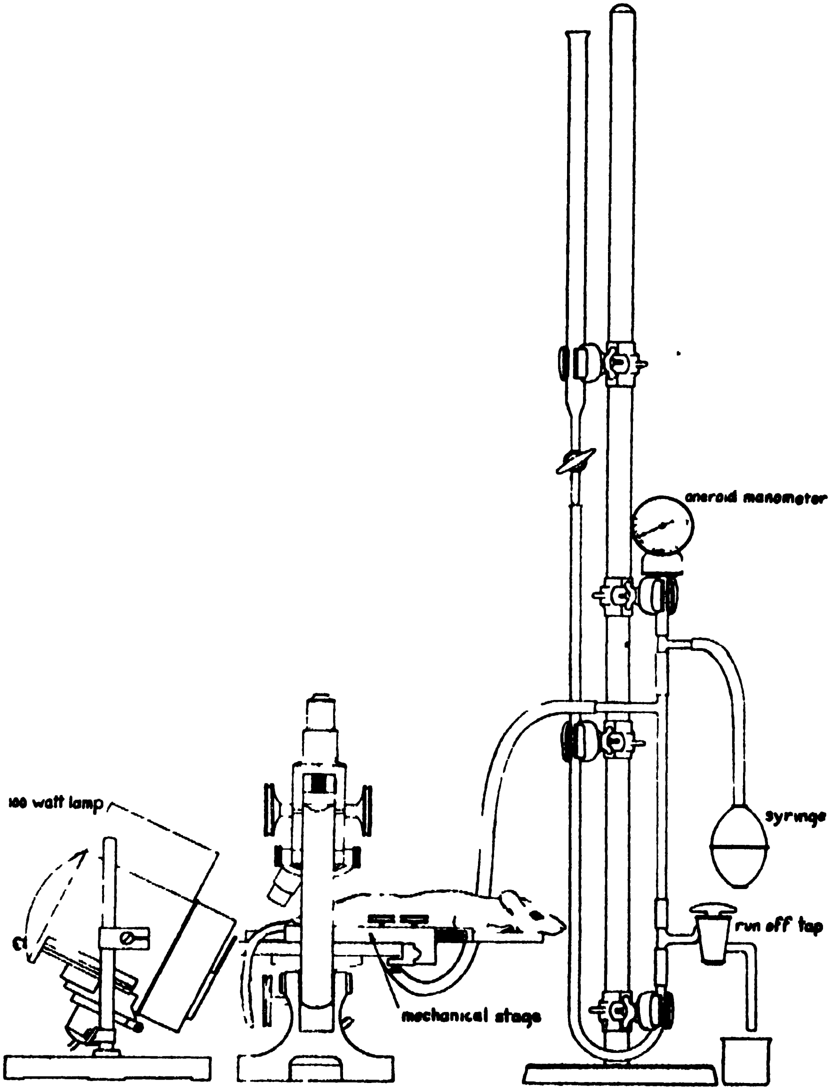


FIGURE 1.—Apparatus for determining systolic blood pressure of the rat (indirect method using the foot).

were held in position by plasticene. After a little practice, and with the aid of a moveable stage, a suitable area was found in which the flow in the fine capillaries could be observed clearly. Then the cuff around the thigh was inflated by means of the rubber syringe until a pressure of 90 to 100 mm. Hg was recorded. Thereafter the pressure was adjusted upwards by increasing the head of Hg until the blood flow in the capillaries stopped. The manometer was read at this point and the pressure observed was recorded as the BP. The pressure in the cuff was immediately re-

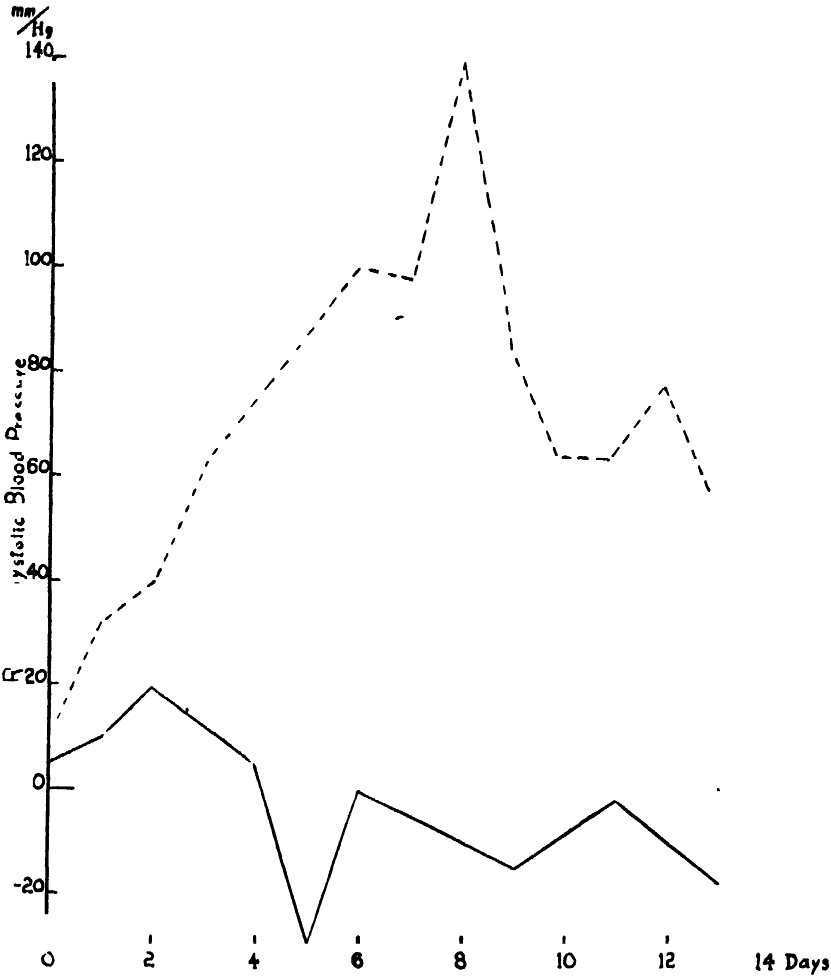


FIGURE 2.—(*a*) Effect of 1 mg. injection of desoxycorticosterone on day 0 (7 rats); (*b*) daily feeding of histidine and ascorbic acid starting with injection as in (*a*) (2 rats).

leased. After a minute or two a second determination was made. Such readings were usually less than 5 mm. apart. If they differed by more than 10 mm., a third reading was taken and the average of the two highest accepted, in accordance with the procedure adopted by Griffith and Farris (1942) and Sulkin and Brizzee (1947). The dosage of sodium pen-

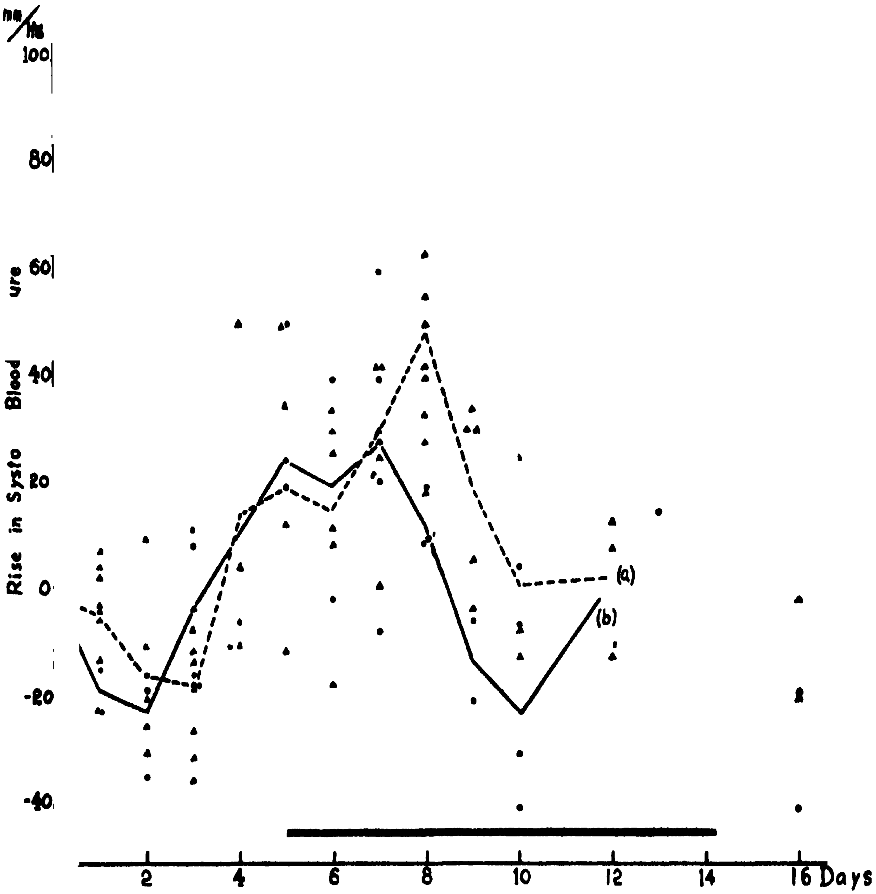


FIGURE 3.- The combined results of Runs I, II and III:

(a) ------ Injected with 1 mg. desoxycorticosterone (day 0).

(b) - - -as in (a) plus daily feed of histidine and ascorbic acid during period marked: ▬

tothal was established for each rat and the "normal" systolic pressure for each determined. Hypertension was induced by a single intramuscular injection of 1 mg. DCA (supplied through the courtesy of Ciba Limited). The hypertensive effect of DCA was counteracted by adding daily to the diet 20 mg. histidine and 70 mg. ascorbic acid. This ameliorating action of histidine and ascorbic acid was reduced or nullified by intramuscular injection of 0.0125 mg. of the antihistamine preparation called antistine (also supplied by Ciba Limited), provided the latter was given within twenty-four hours prior to the next BP determination.

RESULTS

In Fig. 2 is shown the rise in BP above the normal levels for 4 male and 3 female rats after injecting intramuscularly on day 0 1 mg. DCA. The peak was reached on day 8 when the BP had climbed 140 mm. Hg over the normal levels. In the lower curve is seen the effect on 2 rats (male and female) treated similarly except that 20 mg. histidine and 70 mg. ascorbic were added to the diet daily from day 0.

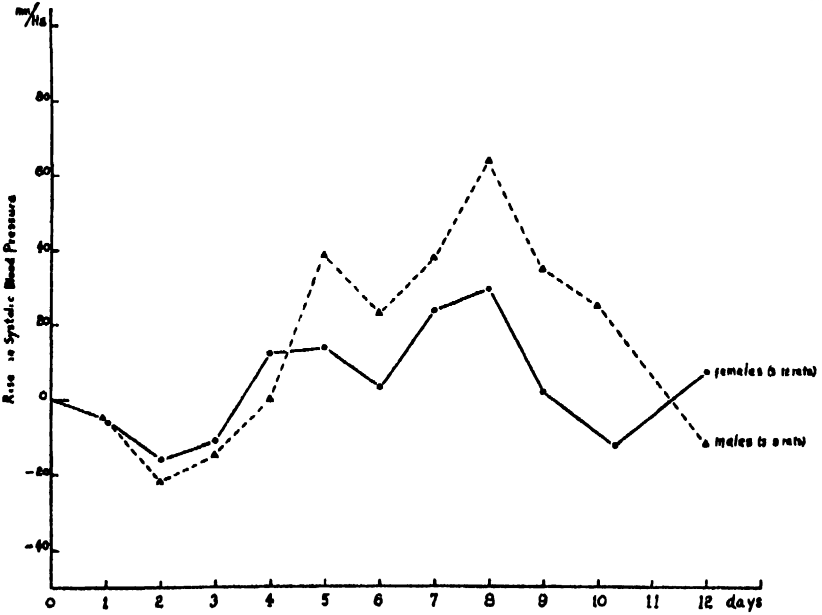


FIGURE 4.—The influence of sex on the experimental hypertension induced by injection of 1 mg. desoxycorticosterone on day 0.

Males - - - Δ - - - Females —•— -

In Fig. 3 is shown the effect of similar treatment with DCA about one year later on the BP of another group of 4 males and 6 females. The lower curve (*b*) shows the results of adding the histidine and ascorbic acid supplement to the daily diet of 3 males and 2 females from day 5 after some hypertension had been induced. The day 8 peak was not reached in this case, and the BP fell to normal or subnormal levels. Both groups showed an initial dip before the subsequent rise in BP.

In Fig. 4 is shown the difference in response of 9 males and 12 females, as far as BP is concerned, following injection of 1 mg. DCA on day 0. Both sexes exhibited an initial dip with a subsequent rise to a peak on the day 8 with the males showing the greater dip and greater rise.

In Fig. 5 each of 4 male and 4 female rats was given 1 mg. DCA on day 0. After an initial dip, the BP rose to hypertensive levels by day 5. At this stage the rats were divided into three groups: (*a*) the BP of 1 male and 1 female given no further treatment rose to a peak on day 8;

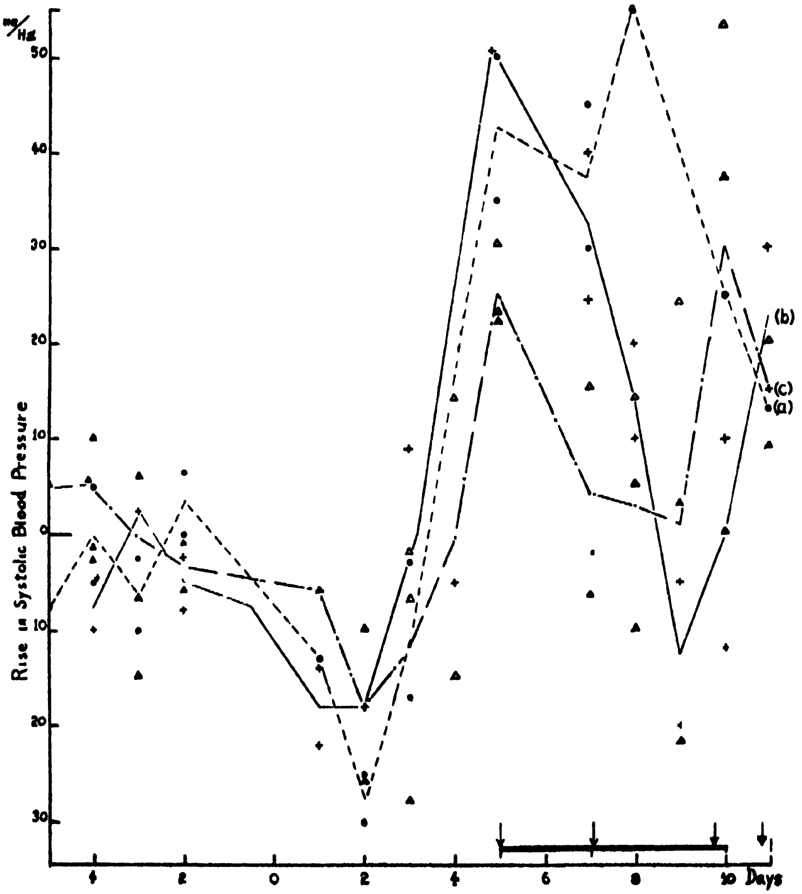


FIGURE 5. -Results of Run II. All rats injected intramuscularly with 1 mg. desoxycorticosterone acetate on day 0.

(*a*) --•---- no further treatment. (1♂, 1♀).

(*b*) —+——70 mg. ascorbic acid and 20 mg. histidine daily in food during period marked▬▬▬ (1♂, 1♀).

(*c*) —·Δ—· as in (*b*) plus intramuscular injections of .05 mg. (↓) or .0125 mg. (↓) antistine (2♂, 2♀).

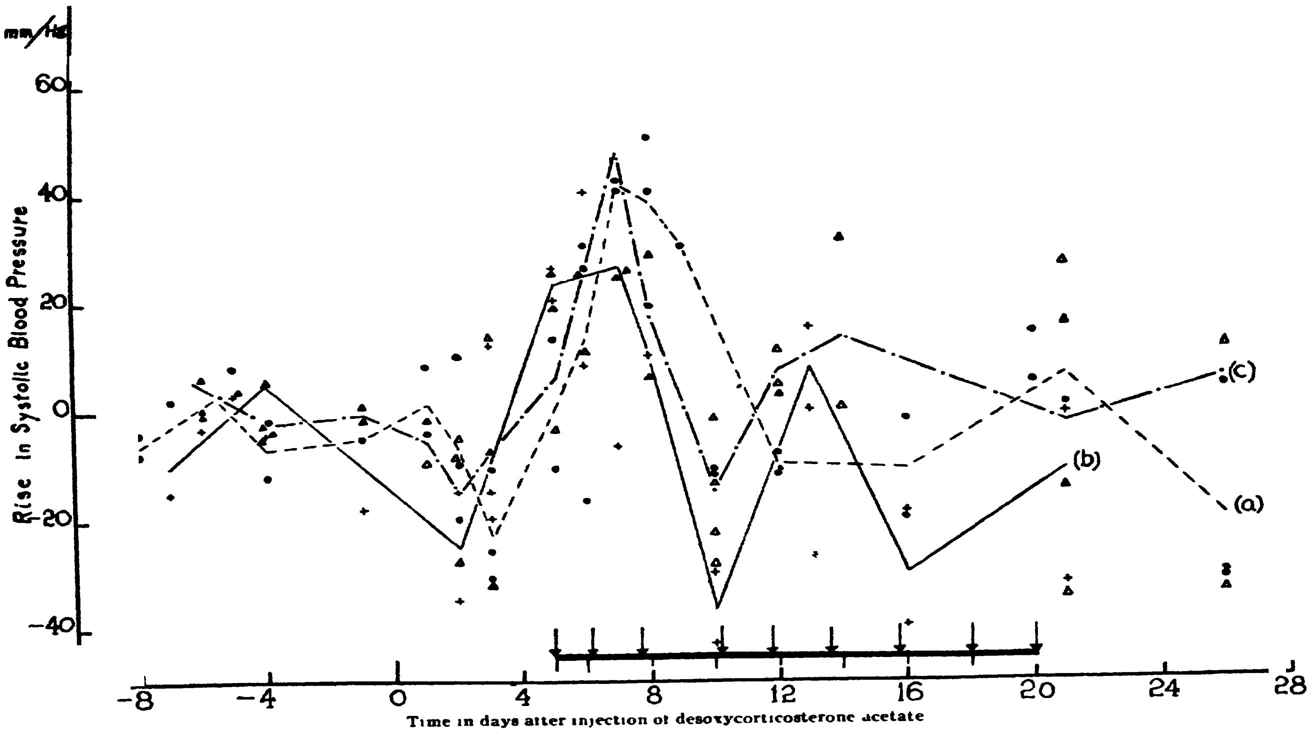


Figure 6.—Results of Run III. All rats injected intramuscularly with 1 mg. desoxycorticosterone acetate on day 0.

(*a*) ---•--- no further treatment (1♂, 2♀).

(*b*) —+——70 mg. ascorbic acid and 20 mg. histidine in food during period marked: ▬▬▬ (2♂, 1♀).

(*c*) —Δ— ·—as in (*b*) plus intramusular injections of antistine at times shown by arrows: ↓ ↓ (2♂, 2♀).

(*b*) 1 male and 1 female from day 5 on received histidine and ascorbic acid daily, and their BP dropped without reaching the day 8 peak; (*c*) 2 males and 2 females from day 5 on received histidine and ascorbic acid daily, and in addition, on days 5 and 7 were injected intramuscularly with .05 mg. antistine which they did not tolerate well. On days 7, 8, and 9 their BP had dropped to approximately normal. The third and fourth antistine injections were reduced to .0125 mg. and given on days 10 and 11, approximately four hours before determining the BP. Toleration of the smaller dosage was much better and the BP was up, approximating that of the other two groups.

In Fig. 6 the action of antistine was further investigated using the smaller dose. Starting with 5 male and 5 female rats, each was given DCA on day 0. Following the initial drop there was a rise in BP. On day 5 the rats were divided into three groups as in Fig. 5: (*a*) 1 male and 2 females without further treatment showed a peak in BP on day 7; (*b*) 2 males and 1 female from days 5 to 20 received histidine and ascorbic acid supplements daily causing the BP curve to flatten out and drop irregularly; (*c*) 2 males and 2 females received the same daily supplements of histidine and ascorbic acid as (*b*) and in addition the antistine injections as shown. As a result (*c*) showed a BP peak on day 7 as did group (*a*).

In Fig. 7, the solid line shows the lowering effect on BP when 3 male and 3 female rats were treated with antistine only, and the dotted line (taken from Fig. 6 group (*c*) for comparison) shows the elevating effect on BP when, five days after injection of DCA, daily supplements of histidine and ascorbic acid were added and antistine injections given at the times (indicated by arrows) which were the same for both groups.

DISCUSSION

All determinations of BP were made in duplicate. Good agreement was obtained between the duplicates of any pair. For example, of the seventy-three pairs of readings made for Fig. 5, only two pairs showed a deviation of more than 10 mm. Hg, while six others showed a deviation of exactly 10 mm. and the average deviation was only 4 mm. Hg. Deviations in paired readings were similar for treated and untreated rats.

The method used in determining BP in the rat has given normal values ranging from 106 to 145 mm. Hg with an average value of 127. A slight but definite rise in BP was noted with increase in age. No appreciable difference in the BP of the two sexes was noted, nor was any detected for the different phases of the sex cycle. Changes in BP with time of day were negligible. Such findings agree closely with those of other workers.

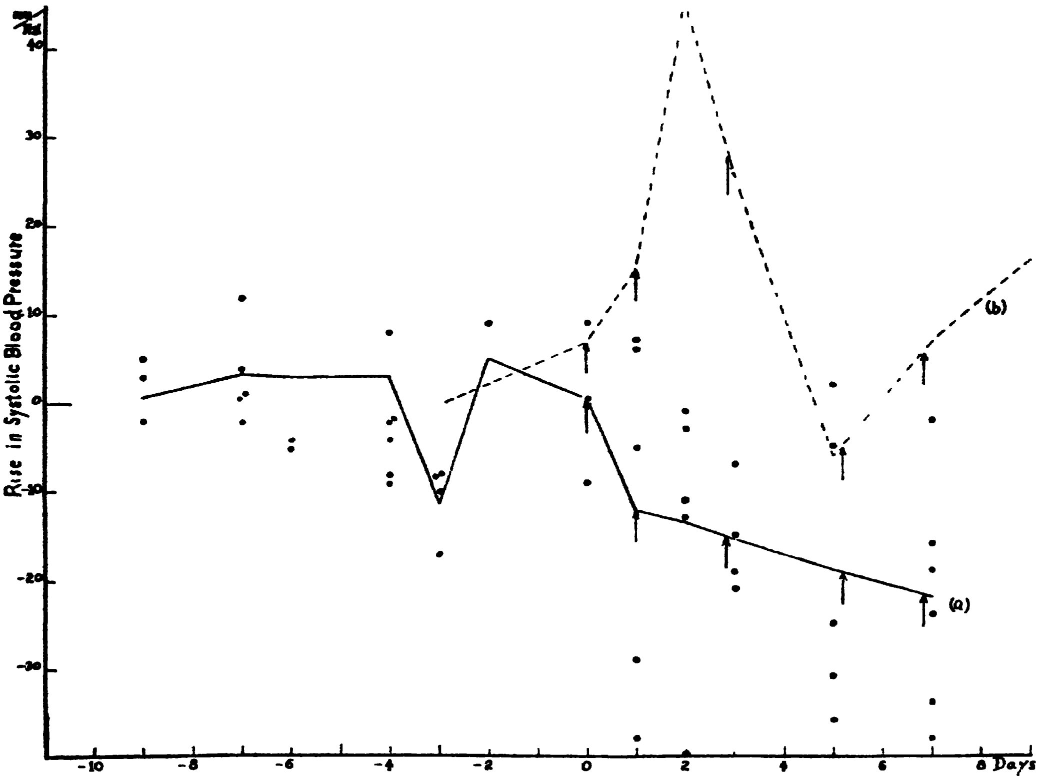


FIGURE 7.—Results of Run IV, showing the effect of (*a*) .0125 mg. intramuscular injections of antistine at times shown by (↓) compared with (*b*) similar injections of antistine on rats in group (*c*) of Run III (Fig. 6). First antistine injection on day 0 in both cases.

Since BP in this work was measured under anaesthesia, the values obtained are open to the criticism of Sulkin and Brizzee that anaesthesia lowers the BP from 7 to 61.5 mm. Hg. Their report does not include sodium pentothal but does include other barbiturates such as nembutal and amytal, for which they reported depressions of 20.7 and 21.3 mm. Hg respectively. However, since our figures agree with those obtained by Sulkin and Brizzee for unanaesthetized rats, and since Pratt, Tatum, Hathaway, and Waters (1936) have reported that, under light anaesthesia with pentothal, BP usually falls immediately after the anaesthetic is given but generally returns quickly to the previous level, it was felt that under the procedure adopted the BP values obtained by us represent the normal systolic pressures.

The effect on BP of 1 mg. DCA given intramuscularly, as found separately by Fitch and by Semple and shown respectively in Figs. 2 and 3, was a marked rise on the seventh or eighth days. The degree of elevation, however, was different, Fitch getting an average rise of 140 mm. Hg while Semple obtained an average rise of 50 mm. Hg. Prado, Dontigny, and Selye (1947) have reported elevations of 150 to 160 mm. Hg above normal but these are the result of subcutaneous implants of 40 mg. pellets of DCA. Further, Semple has found an initial drop in BP followed by the rise, while Fitch got no initial drop.

The difference in degree of response to DCA depending on the sex of the rat may be significant. Since progesterone secretion is more characteristic of the female than the male, and since progesterone and DCA show considerable chemical and certain physiological similarities, there is the possibility that the female already handling greater amounts of progesterone will show the less marked effects on the injection of DCA.

Figs. 2 and 3 clearly indicate that histidine and ascorbic acid, given along with DCA, can prevent the development of hypertension due to DCA, or, if given after hypertension has developed, can ameliorate it. Separately histidine and ascorbic were ineffective. Fitch also found that when histidine and ascorbic acid were given daily to otherwise untreated rats the BP dropped an average of 13 mm. Hg below the normal values.

Since histidine, by decarboxylation without deamination in the colon, can form histamine, and since histamine, which is normally destroyed through oxidation by histaminase, is not likely to be destroyed as readily in the presence of a reducing agent such as ascorbic acid, any vasodilatory action and consequent lowering of the BP due to the presence of histamine is more likely to occur when supplements of histidine and ascorbic acid are added to the diet. The action of the antihistamine substance (antistine), as shown in Figs. 6 and 7, would lend support to this explanation of the amelioration of hypertension with histidine and ascorbic acid. In other words, this amelioration was inhibited by antistine, provided the BP determination was made within twenty-four hours after giving the water-soluble antistine. When the dosage of antistine was large (first two injections in Fig. 5), the BP dropped rather than rose as had been expected. With smaller doses of antistine the BP rose (Fig. 6). An explanation of this appeared (Fig. 7) when small doses of antistine alone were given to untreated rats and caused a lowering of the BP. Apparently the antistine not only has an antihistaminic action, permitting the DCA to exert its pressor effect but, when given to untreated rats, exerts an independent depressor action.

SUMMARY

Hypertension has been induced in albino rats by intramuscular injection of 1 mg. DCA, the peak in the BP curve coming on the seventh or eighth day and hypertension lasting from one to two weeks.

Male rats showed a greater response to DCA than did females.

The action of DCA in producing hypertension was prevented when both histidine and ascorbic acid were fed, but not by either alone. Histidine and ascorbic acid were also effective in ameliorating hypertension already developed by DCA, or in lowering the normal BP.

The countering effect of histidine and ascorbic acid on the hypertensive action of DCA was in turn destroyed by the action of antistine.

REFERENCES

ABRAMMS, M. and SOBIN, S. S. (1947). Proc. Soc. Expt'l Biol and Med., **64**: 412.

GOLDBLATT, H., LYNCH, J., HANZAL, R. F., and SOMMERVILLE, W. W. (1934). J. Expt'l Med., **59**: 347.

GRIFFITH, J. Q. and FARRIS, E. J. (1942). The rat in laboratory investigation. Philadelphia.

GROLLMAN, A. (1946). J. Amer. Dietetic Assoc., **22**: 864.

KING, A. L. (1947). Science, **105**: 127.

KNOWLTON, A. I., LEOB, E. N., STOERK, H. C., and SEEGAL, B. C. (1947). J. Expt'l Med., **85**: 187.

PAGE, H. I. (1939). J. Amer. Med. Assoc., **133**: 604.

PRADO, J. L., DONTIGNY, P., and SELYE, H. (1947). Proc. Soc. Expt'l Biol. and Med, **66**: 446.

PRATT, T. M., TATUM, A. L., HATHAWAY, H. R., and WATERS, R. M. (1936). Amer. J. Surg., **31**: 464.

SCHAFER, P. W. (1944). Surg., Gynecol. and Obstet., **79**: 163.

SULKIN, N. M. and BRIZZEE, K. R. (1947). Proc. Soc. Expt'l Biol. and Med., **64**: 125.

THOMAS, C. B. (1947). Bull. John Hopkins Hosp. Cited by Wiggers in Ann. Rev, Physiology, **9** (1947): 289.

WIRTSCHAFTER, Z. T., and WIDMAN, R. (1947). J. Amer. Med. Assoc., **133**: 604.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Measures of Abundance in Dog-fish (*Squalus suckleyi*)

By W. E. BARRACLOUGH

Presented by J. L. HART, F.R.S.C.

AS the requirements for vitamin A increased during World War II, the livers from the Pacific Coast dog-fish (*Squalus suckleyi*) became one of the principal sources of vitamin A. In British Columbia there has been a fairly intensive fishery for the dog-fish or grayfish during recent years. Under the pressure of a high and increasing fishing intensity, the catch of this shark in British Columbia waters dropped greatly during the years following 1944. The decline in the total landings of dog-fish livers is indicated in Table I.

TABLE 1

QUANTITY OF DOG-FISH LIVERS LANDED IN BRITISH COLUMBIA FROM 1937 TO 1946

	Weight of dog-fish livers landed in lb.
1937	178,900
1938	933,300
1939	308,047
1940	1,566,500
1941	3,552,576
1942	4,241,256
1943	5,121,186
1944	7,769,564
1945	5,821,849
1946	2,844,217

Exploitation of any virgin fishery tends to change the abundance of the fish population on which the commercial yield depends. Effective fisheries administration depends upon knowledge of the extent of the exploitation and its course. In general, direct measures of fish populations are not feasible, but relative estimates can be obtained from analyses of fisheries statistics. As a rule, such statistics are most useful when they include detailed accounts of the amount of fishing effort and the localities fished. In most fisheries total catch is too involved with the amount of fishing effort to reflect well the changes in the abundance of fish on the grounds. Calculation of the return per unit of fishing effort is one of the most valuable indices of availability and abundance. Should the amount of effort expended vary from year to year, catch per unit of effort cannot be used directly to indicate abundance without considering the actual amount of effort expended in successive years, because of the influence of competition for fish between units of gear. Conclusions can be distorted by cumulative and unassessable changes in the quality of the effort. Other measures have been employed to indicate the availability or relative abundance of a population.

In this study availability refers to the relative amount or apparent amount of dog-fish in the waters of Hecate Strait subjected to the sunken gill-net fishery. Availability in this sense does not mean abundance. Dog-fish may be abundant, that is, these sharks, may be present in great numbers in the waters, but they may not be available to the sunken gill-nets. Availability, therefore, is a relative term applying only to that part of the total population within the horizontal and vertical range of the sunken gill-nets and moving around actively enough to become enmeshed in them.

MATERIALS AND METHOD

Changes in the availability or relative abundance were determined from the analysis of individual tally slips or fish receipts of each fisherman's landing of livers from dog-fish caught by sunken gill-net boats operating in western Hecate Strait and recorded by the fishing industry. In Hecate Strait the sunken gill-net fishery commences in May and usually terminates at the end of October. The fishing grounds are situated in an area off the east coast of the Queen Charlotte Islands, extending from Rose Spit in the north to an area south of Cumshewa Inlet (Fig. 1). The data used in the analysis were compiled from tally slips of the landings of dog-fish livers made by sunken gill-net boats fishing in this area only. Using the method of link relatives, a comparison was made between the average catches per boat per month by the same sunken gill-net boats fishing in consecutive years. The catches of boats fishing in 1943 were compared with those of the same boats fishing in 1944; those of boats fishing in 1944 were compared with those of the same boats fishing in 1945; and the same comparison was made for the years 1945-6. This method of estimating availability has been employed in various fishery investigations. Link relatives are determined by comparing the catch in one month with the catch in the same month in the previous year, or in each succeeding year. In the analysis presented here the year 1943 was used as the base year. Each month of this year was compared with the same months in each of the successive years. Chaddock (1925) describes this method of analysis in detail, and it has been used in the

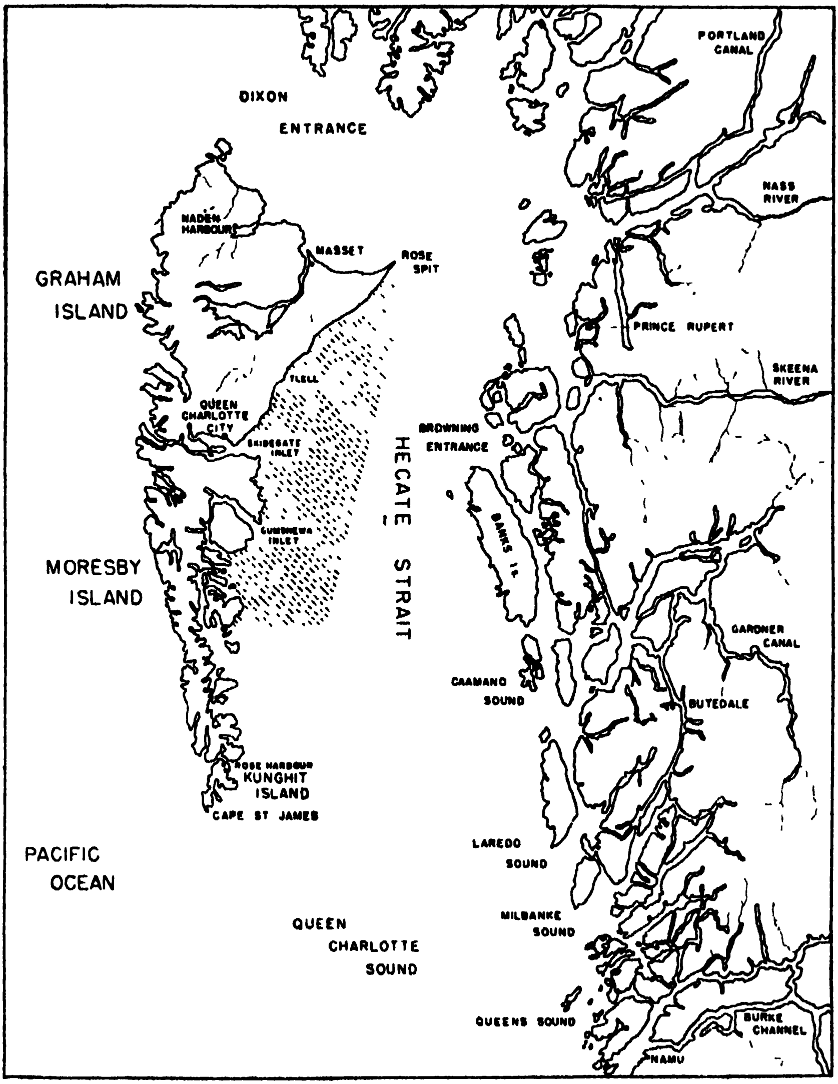


FIGURE 1.—The shaded area indicates the general fishing grounds for the sunken gill-nets in Hecate Strait.

analysis of boat catches in the California halibut fishery (Clark, 1931).

For example: if 8, 12, 6, and 3 are the values of the average catch per boat per month in the successive years 1943 to 1946, the relative for

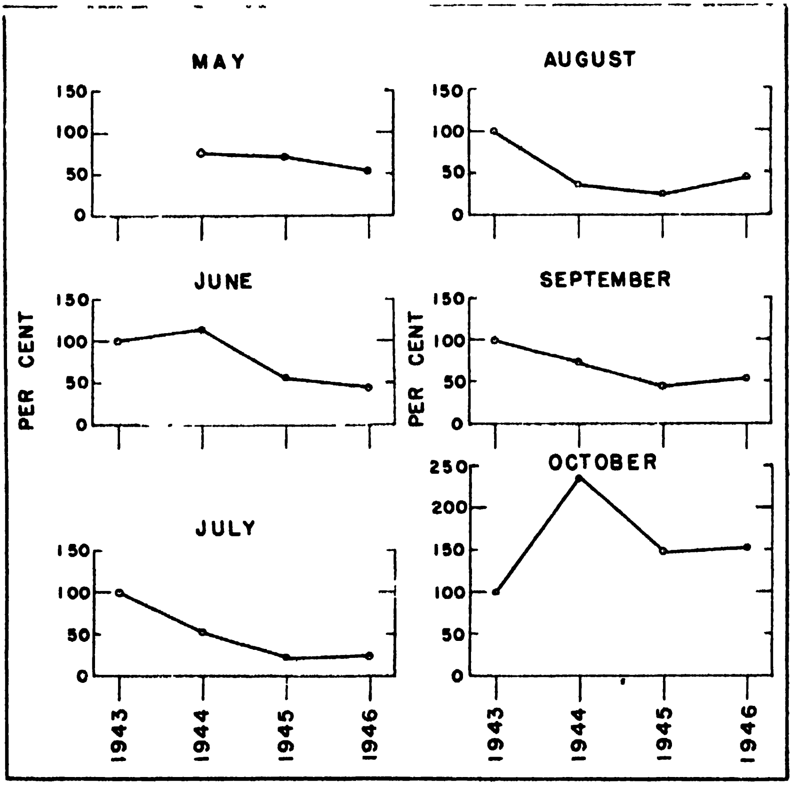


Figure 2.—Index of the catch of dog-fish livers landed by the same sunken gill-net boats fishing in the adjacent years 1943-4, 1944-5, 1945-6, in Hecate Strait. Determined by the method of link relatives.

the second year (1944) using the first year (1943) as a base, would be $12 \div 8 = 1.50$; the relative for the third year would be $6 \div 12 = 0.50$, and the relative for the fourth year (1946) would be $3 \div 6 = 0.50$. These relatives must now be chained together. The first year of the series, 1943, is the base year and is therefore 100 per cent. The chain relative for 1944 would be 100 multiplied by $1.50 = 150$ per cent; the chain relative for 1945 would be $150 \times 0.50 = 75$ per cent; and the chain relative for 1946 would be $0.75 \times 0.50 = 37\frac{1}{2}$ per cent. The chain relative for the years 1944 to 1946 would then be 1.50, 0.75, and 0.375.

DATA AND DISCUSSION

The index of the landings of dog-fish livers each month made by the same sunken gill-net boats fishing in the consecutive years 1943-4, 1944-5, 1945-6 in Hecate Strait as determined by the method of link relatives is shown in Fig. 2. Fig. 2 indicates that there has been a decline in the availability of dog-fish for each month, except October, from 1943 to 1946. This graphic presentation does not permit comparison of the availability in one month with the availability in another month in the same year. Availability may only be compared for each month separately for the years 1943 to 1946. When the total landings of dog-fish livers from the same sunken gill-net boats fishing in consecutive years are linked together by the method of chain relatives a clearer picture of the availability or relative abundance of the dog-fish in Hecate Strait from 1943 to 1946 is demonstrated (Fig. 3). The index of the availability drops from 1943 to 1945 and then increases slightly in 1946.

In addition to the decline in availability of dog-fish and the total landings of livers from Hecate Strait from 1944 to 1946, there has been a noticeable decline in the vitamin A potency of the dog-fish livers from 1944 to 1947. The average vitamin A potency of livers from dog-fish caught in the same area in Hecate Strait by sunken gill-net boats only has declined with each succeeding year since 1944. The weight of livers landed from dog-fish caught in Hecate Strait in 1947 would represent a lower stock of vitamin A than the same weight of livers recovered from dog-fish caught by the same gear and in the same area in 1944. The decline in potency of vitamin A is indicated in Table II.

TABLE II

	Average number of USP units per gram of liver oil	*Total poundage of dog-fish livers tested*
1944	14,648	148,191
1945	13,183	514,000
1946	9,784	446,448
1947	9,075	572,486

The average number of USP units per gram of liver oil was obtained from livers removed from dog-fish caught by sunken gill-nets only in Hecate Strait near the Queen Charlotte Islands. The average number of USP units was determined from daily liver oil analyses made available by the fishing industry, which included as many separate landings of dog-fish livers as possible. Livers tested for vitamin A as shown in

Table II were landed from the beginning of May until the end of October in each year.

Vitamin A concentrations in dog-fish livers are known to be related to other readily observable characteristics. They are positively correlated with body length (Pugsley, 1937a) and presumably through length with age. There is also a close association between sexual maturity

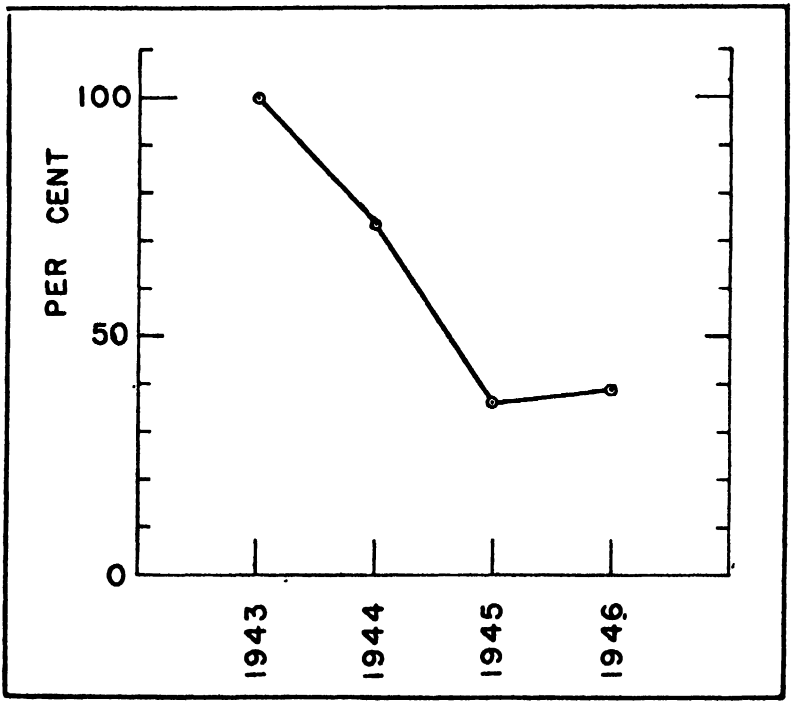


FIGURE 3.—Index of the total catch of dog-fish livers landed by the same sunken gill-net boats fishing in the adjacent years 1943-4, 1944-5, 1945-6 in Hecate Strait. Determined by the method of link relatives.

of the dog-fish and the vitamin A concentration of the livers. Livers from immature dog-fish contain only a small percentage of vitamin A as compared with that found in the livers of the larger fish. The vitamin A content of the darker coloured livers, which are found mostly in the larger male and female dog-fish, is higher than that of the lighter coloured livers found in the smaller dog-fish (Pugsley, 1937b). Sanford and Bonham (1946) indicate that age is probably the determining factor in vitamin A production in the livers independent of the length of the dog-fish. They found that within a length frequency category there was an inverse relationship between liver colour and the average posterior spine length of the dog-fish. It was assumed that for dog-fish of the same size and sex the fish with the darker livers are probably the older since their blunt spines would have been subjected to wear for a longer time. This would indicate that the darkest livers with the highest vitamin A concentrations are found in the slower growing dog-fish.

The sunken gill-net fishery is rather a selective one for size. Only the largest fish are caught, the smaller ones tend to pass through the webbing, and thus those of no commercial value are eliminated. The decline in availability and total catch would indicate that the larger fish are becoming less abundant on the fishing grounds in Hecate Strait. The consistent falling off in vitamin A potency suggests that the older dog-fish in the size groups selected by the sunken gill-nets have declined more rapidly than the younger ones, and that the fishery is relying to an increasing extent upon the faster growing, moderately old individuals. As the older age classes of dog-fish are removed from the population, the number of larger dog-fish with livers of a high vitamin A potency available to be caught in sunken gill-nets becomes less. This situation is not surprising in a species which has been fished so aggressively and so intensively throughout its range and against whose largest members a special fishery has developed. Such a selective fishery, is dependent upon the older fish, is probably necessary to secure the maximum production from the fish population. It cannot be considered that the decline in availability of the dog-fish and the drop in vitamin A potency of the dog-fish livers are sufficient to indicate biological depletion. The economic condition in which reduced yield per boat is accompanied by reduced value per unit is quite different and is a matter of real concern.

REFERENCES

Chaddock, R. E. (1925). Principles and methods of statistics. Cambridge, Mass., Houghton Mifflin.

Clark, G. H. (1931). The California halibut (*Paralichthys californicus*) and an analysis of the boat catches. Calif. Div. Fish and Game, Fish Bull. no. 32.

Pugsley, L. I. (1937a). Variations in the vitamin A content of the liver oil of the greyfish (*Squalus sucklii*). Fish. Res. Board of Canada, Prog. Rep. Pac., no. 31, pp. 3-5.

————(1937b). The vitamin A content of grayfish liver oil (*Squalus sucklii*). Fish. Res. Board of Canada, Prog. Rep. Pac., no. 34, pp. 3-7.

Sanford, F. B. and Bonham, K. (1946). Grayfish liver colour related to fin-spine length. Com. Fish. Rev., 8: 6-8.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

The Sympathicotropic, Interstitial, and Phaeochrome Cells in the Human Foetal Testis

By LOUIS BERGER, F.R.S.C.

THE behaviour of the sympathicotropic cells (to be called S-cells in this paper) in the ovaries in foetuses and at birth, their similarities with ovarian interstitial and thecal cells, and their fundamental discrepancies with phaeochrome elements were reported to this Society in 1945. In view of the intricate histological features prevailing in foetal ovaries and obscuring some details, it was thought that an inquiry into the corresponding, more clear-cut, testicular structures might not only confirm the results obtained in the study of foetal ovaries, but also yield additional information about the topographical conditions of the respective elements and eventually about the histogenesis of the S-cells.

Twenty-one pairs of male gonads from embryos of 28 mm. C-R length up to foetuses of eight and one-half months, at half-monthly intermediate stages, were examined either in total serial sections or in series of ten-section intervals. For some stages two or three pairs of glands were available. Most glands were cut in longitudinal sections so as to show at the central levels at the same time the testis, at least part of the head and tail of the epididymis, and the ductus deferens with its surrounding tissue containing the vessels and nerves.

In the actual description only the interstitial, sympathicotropic, and phaeochrome cells and their topographical features will be considered and their eventual similarities and/or discrepancies discussed.

Interstitial cells (i.c.) are already present in testes of 28 mm. C-R length. At that stage they show an indistinctly outlined, homogeneous, and pale protoplasm which may emit short expansions. In the following stage of 32 mm. the i.c. become rounded and the protoplasm of some is divided into a narrow transparent or vacuolated outer zone, and an inner compact slightly basophilic zone, with a few small round or slightly elongated inclusions or condensations at the junction of the two zones. At 45 mm. protoplasmic condensations are still more pronounced and may be grouped in circles or horseshoe form. These granular or short rodlike protoplasmic inclusions, either scattered or grouped as just mentioned, become a characteristic feature of the i.c. in the following stages of 55, 67, 115, 140 and 150 mm., where they are present in many cells. During these stages, the bulk of the i.c. surpasses that of the sex cords. From 150 mm. on, their relative number shows a slow decrease, and the protoplasmic condensations are also present in fewer cells, although they may occasionally be found up to the time of birth. It must, however, be emphasized that there seem to exist individual variations, for in some glands of the same age the number of i.c. and their protoplasmic features may be different; in one case of our series such discrepancies were even encountered between the two testes of the same foetus. No i.c. were ever found outside the territory of the testis proper.

Up to the stage of 115 mm. C-R length, no S-Cells could be found in any nerve running along the ductus deferens or the testis. At this stage one isolated, large, pale, and slightly angular cell was present almost in the centre of a nerve, between its fibres. No similar cells were seen in glands from foetuses of 140 and 142 mm., but a few were present in a foetus of five months (150 mm). From that time on, they became conspicuous, although they, too, showed individual fluctuations, being very numerous in glands of six months, but almost scarce in others of six and one-half months; in three pairs of glands of seven months one showed abundant cells in one testis and only a few in the other, in the second pair the S-cells were moderately numerous, and in the third scarce, whereas in a testis of a foetus of seven and one-half months they were more numerous than in all others.

All these cells lie either singly, or in small groups, or in short or long rows between the inner nerve fibres or beneath, in place of, or immediately outside of the perineurium. Their nuclei show a fine chromatin network and a distinct, moderately large nucleolus or sometimes two or three nucleoli, but the form may be either regular (round or oval) or irregular (more or less elongated and anfractuous or even shrivelled). The protoplasm is still more polymorphous and almost defies any standard description: some S-cells are the size of large interstitial cells or larger, with a finely granular protoplasm around the nucleus and a clear or empty peripheral zone and a few coarse granular or short rodlike condensations lying mostly at the junction of the two zones similar to those in some interstitial cells, but scarcer; some are smaller and show a uniform protoplasm; some are almost entirely vacuolated; some are still smaller, with their protoplasm, either pale or dense, reduced to a very small rim. The latter look like atrophic or involuting elements, and in some places S-cells may be difficult to distinguish from endoneural cells.

The S-cells are located principally in one nerve and its branches which run along the ductus deferens between its many vessels and extend along the testis between the latter and the ductus epididymidis, bending finally around the lower pole of the testis, away from the tail of the epididymis towards the albuginea. S-cells may be present in the nervus ductus deferens at a level as far as 8 mm. from the nearest border of the testis, and then all along that nerve and its branches, including the region of the hilus, up to where the diminished nerve bends into the albuginea. No S-cells were found in the few branches which could be followed into the testis proper, nor could any continuity be seen between interstitial and S-cells.

Although S-cells were found all along this nerve, they were as a rule much more numerous along the ductus deferens than nearer the testis, and in many cases they seemed to appear first at levels farther away from the testis.

Phaeochrome elements were found in six cases of the actual series, the first at the stage of 80 mm. C-R length, the last at eight and one-half months. They form oval or elongated structures, either very small or visible to the naked eye (the largest measured 0.4 x 4.0 mm.), which lie without exception very close to the paradidymis, that is, always some distance from the testis along the ductus deferens. The bodies are as a rule appended to one or two small nerves which may be found running tangentially to the bodies or apparently stopping at their border with no visible fibrils between the alveoli or the individual cells. The cells are grouped in small compact alveoli of a few elements; between the alveoli run capillaries which are often distended and form a dense network. The cells are moderately large and rather regular, either round or approximately oval. Their nuclei show a pulverulent chromatin, mostly without any conspicuous nucleoli or with one or more very small granular condensations, very different from the nucleoli of S-cells. After Bouin fixation the cells look very vacuolar, "washed out," with very indistinct outlines and a protoplasm reduced to pale debris; sometimes, however, the cells are neatly delimited and show a pale protoplasm of varying density. After Regaud fixation or similar dichromate solutions, some or almost all cells show a distinct brown tint, becoming green after Mann's stain. Even in cases where the tissues have been fixed without dichromate or where the cells are not yet distinctly phaeochrome, their nature appears after silver staining following the Rogers method; indeed, the phaeochrome cells are distinctly argentophile, and, although this reaction is not in itself specific and absolutely selective, it is of primary significance in the present case, because it is conspicuously negative in interstitial and S-cells, a fact definitely established by van Campenhout.

The features may, therefore, be summarized as follows: during foetal life, the interstitial cells are very abundant up to six months, when they begin slowly to decrease; the first S-cells were found in a foetus of 115 mm., but are conspicuous only from the sixth month onwards, when they become a regular constituent of the gonads. Phaeochrome bodies were only present in six of twenty-one pairs of testes, one as early as at 80 mm. C-R length.

As to topography the i.c. were confined to the territory of the testis proper; the S-cells were always in close relation with nerves, lying between the fibres or beneath, in place or around the perineurium, and were found all along the ductus deferens, up to 8 mm. from the testis, and along the latter. The phaeochrome bodies were in the present series restricted to the area of the paradidymis, always more or less distant from the testis.

The S-cell clusters were almost always pervaded by more or less numerous nerve fibres, whereas the phaeochrome bodies were only appended to nerves. The cells of the latter were regularly argentophile after Rogers' silver method, which always left the S-cells unstained.

The morphological features of the three cell-species differed from each other, although some characteristics, like the protoplasmic condensations, were common to interstitial and S-cells. Thus, contrary to what is seen in testes of adults, the foetal S-cells could not be identified with the i.c. of the corresponding ages and no continuity was ever found between the two cell-species.

It must, therefore, be concluded that the S-cells arise independently from the i.c., and the available evidence suggests that they originate in the places where they are seen. This raises the question of their cellular origin or derivation. It is obvious that the constant intranervous or immediately perinervous situation of the S-cells can only allow for a descent either from Schwann cells or from cells of the endo- or perineurium or eventually from both. The frequent simultaneous presence of S-cells between the nerve fibres and in place of and/or outside of the perineurium, suggests a priori an origin from endo- and perineural cells, which are essentially the same cells, whatever may be their ultimate connective or nervous derivation. Another inferential argument in favour of this origin is the fact that no S-cells were found at stages or in gonads where the nerves did not yet show an endoneural septation. The appearance of unmistakable endoneural cells is very unequal in the various nerves going to the testis and its appendages, and may even vary at different levels of the same nerve, but as a whole becomes conspicuous during the fifth foetal month, thus preceding only shortly, if at all, the appearance of the S-cells.

The features of the S-cells in foetal testes appeared, thus, to be unexpectedly different from their counterpart in foetal ovaries and from those in adult testes. Indeed, in foetal ovaries, the morphological characters of the S-cells were very similar to those of the interstitial and thecal cells of the same age, and in adult testes the S-cells were identical with the Leydig cells of the same glands, differing only in their nervous connections, but showing often a direct continuity between cells of the two types.

Inasmuch as the i.c. are known to disappear after birth, and as the same seems to be the case with the S-cells on the one hand, and as both cell species are again present in adult testes on the other hand, the histogenesis of the S-cells in adult glands cannot be deduced from the actual results and asks for new investigations before and at the period of puberty.

REFERENCES

BERGER, L. (1945). The sympathicotropic cells in the ovaries of foetuses and newborns. Trans. Roy. Soc. Can., **39**, sec. V: 23.

VAN CAMPENHOUT, E. (1946). Cellules sympathicotropes du testicle et de l'ovarie. Bull. Acad. Roy. de Belgique, 6e série, **11**: 34.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Serum Alkaline Phosphatase and Alloxan Diabetes in Rats*

By MAX M. CANTOR, PERSIS A. WIGHT, and JULES TUBA

Presented by G. HUNTER, F.R.S.C.

INTRODUCTION

A common finding in untreated diabetes mellitus is an increase in serum inorganic phosphorus. One of the factors which determines the level of blood phosphorus is the activity of phosphatases. Therefore it was planned to investigate the activity of these enzymes in relation to alloxan diabetes in rats. This paper deals only with our observations on the alkaline enzyme.

In a preliminary report (Cantor, Tuba, and Capsey, 1947) it was demonstrated that the development of alloxan diabetes in rats is accompanied by an increase in serum alkaline phosphatase, and that the administration of insulin produces a decrease in enzyme activity. Table I provides a record of the characteristic effect obtained.

There is a rapid and progressive rise in blood sugar. This exceeds 450 mg. per cent at the end of two days and is maintained at about this level for many weeks. The alkaline phosphatase shows an initial decline in the first three hours and then increases slowly over the next two weeks until values three times the normal are reached and maintained. The increase in phosphatase activity, like the hyperglycaemia, is readily controlled with insulin; but the effect on phosphatase activity is not as dramatic as it is on the hyperglycaemia. These results are presented graphically in Fig. 1, where the values are calculated as a percentage of the initial level.

METHODS AND MATERIAL

Specimens of tail blood were used for all estimations and collections were made under uniform conditions. These will be described in detail in a separate communication. Blood sugar was estimated by the micromethod of Reinecke (1942). Serum alkaline phosphatase was estimated at pH 9.3 by the method of Shinowara *et al.* (1942) as modified by Gould and Schwachman (1943). Normal values for these are to be reported in a separate paper.

---

*This work is part of Project M.P. 80, conducted with the assistance of a grant to Max. M. Cantor from the Division of Medical Research, National Research Council, Ottawa.

TABLE I

Effect of alloxan diabetes on blood sugar and serum alkaline phosphatase

Time after alloxan	Blood sugar (mg./100 ml.)	Serum alk. phos (units/100 ml.)
0 hrs.	123	100
2 hrs.	166	—
3 hrs	216	56
6 hrs		64
9 hrs.	250	89
12 hrs	310	105
24 hrs.	380	125
2 days	464	154
3 days	440	170
4 days	425	205
5 days	426	223
7 days	358	177
8 days	412	250
14 days	444	365
21 days	420	371
42 days	437	380

Effect of three doses of 0.4 units of insulin at three-hour intervals on blood sugar and serum alkaline phosphatases in alloxan-diabetic rats

Time after insulin	Blood sugar (mg./100 ml.)	Serum alk. phos (units/100 ml.)
0 hrs	425	384
1st dose		
1½ hrs.	150	346
3 hrs.	154	284
2nd dose		
1½ hrs.	77	236
3 hrs.	101	197
3rd dose		
1½ hrs.	51	161
3 hrs.	122	132
9 hrs.	110	206

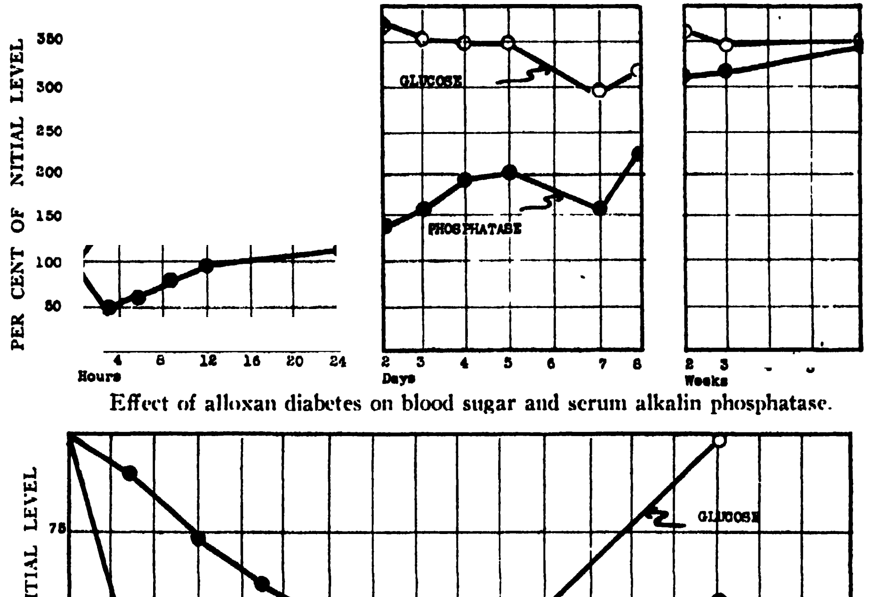
EXPERIMENTAL

The observation that serum alkaline phosphatase increased in nearly parallel fashion with the blood sugar suggested some direct relationship between them and it became necessary to test this hypothesis. With this in view an attempt was made to produce an increase in blood sugar concentration in normal and diabetic rats using parenteral glucose and adrenalin.

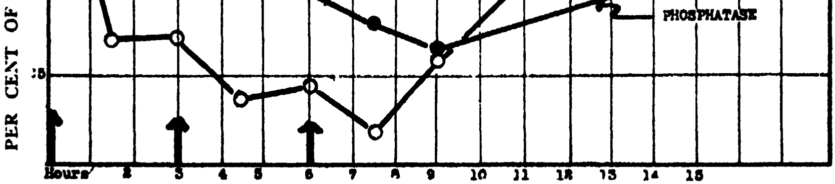
1. *Parenteral glucose*

Following a sixteen-hour fast, six normal male adult rats were given 3.5 gm. glucose per kg. body weight by intraperitoneal injection. Six diabetic rats were given a reduced dose (2.1 gm. per kg.) in similar fashion. Blood sugar and alkaline phosphatase concentrations were followed at frequent intervals for eight and one-half hours. The results are collected in Table II.

FIGURE 1



Effect of alloxan diabetes on blood sugar and serum alkalin phosphatase.



Effect of 0.4 units insulin at 0, 3, and 6 hours on blood glucose and serum alkaline phosphatase in alloxan diabetic rats.

2. *Effect of adrenalin*

The effect of four successive intramuscular injections of 0.2 mg. adrenalin in oil at three-hour intervals on normal and diabetic rats is shown in Table III.

It is evident that both parenteral glucose and adrenalin produce an increase in blood sugar in both the control and diabetic animals. In the con-

TABLE II

Alterations in Blood Glucose, and Serum Alkaline Phosphatase following Parenteral Glucose
Normal rats (3.5 gm. glucose per kg.)
Alloxan diabetic rats (2.1 gm. glucose per kg.)

	Control rats		*Diabetic rats*	
Time after glucose Admin.	Blood glucose (mg. per cent)	Alk. phos. (units per cent)	Blood glucose (mg. per cent)	Alk. phos. (units per cent)
Before fast	130	117	420	284
After fast	106	70	240	114
15 min.	380	—	-	—
30 min.	430	76	303	- -
45 min.	458	—		- -
60 min.	400	60	375	120
2 hrs.	336	57	440	110
4 hrs.	140	53	520	119
5 hrs.	-	—	480	—
5.5 hrs.	100	50	-	—
7 hrs.	—	—	370	105
8 hrs.	—	47	..	—
8.5 hrs.	—	—	344	—

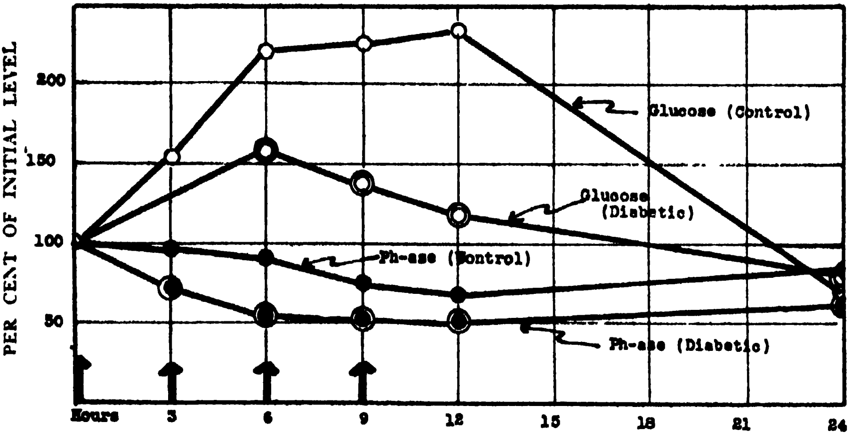
TABLE III

Effect of Four Doses of 0.2 mg. Adrenalin at Three-Hour Intervals on Blood Sugar and Serum Phosphatases

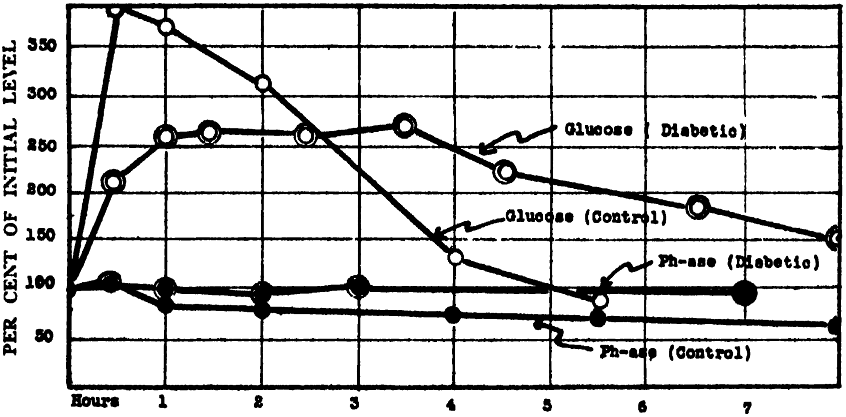
	Control rats		*Diabetic rats*	
Time after adrenalin injection	Blood glucose (mg. per cent)	Alkaline phos. (units per cent)	Blood glucose (mg. per cent)	Alk. phos. (units per cent)
0 hrs.	120	115	360	281
1st dose				
3 hrs.	184	112	560	210
2nd dose				
3 hrs.	270	104	500	158
3rd dose				
3 hrs.	275	87	500	150
4th dose				
3 hrs.	283	83	448	147
15 hrs.	86	98	300	185

trol group receiving glucose, alkaline phosphatase is reduced from a fasting level of 70 units to less than 50 at the end of eight hours. The diabetic group, on the other hand, showed no such reduction. Among the animals receiving adrenalin, both the control group and the diabetic group showed

FIGURE 2



Effect of 0.2 mg. adrenalin at 0, 3, 6, and 9 hours on blood glucose and serum alkaline phosphatase in alloxan diabetic and control rats.



Effect of intraperitoneal glucose on blood glucose and serum alkaline phosphatase in alloxan diabetic and control rats.

reduction in phosphatase activity, this being more marked in the latter. These data are assembled in graphic form in Fig. 2.

These experiments provided evidence against the observations first described, and led to studies on the effect of diet on phosphatase activity.

Effect of Food Intake on Phosphatase Activity

1. *Effect of fasting*

Food was withdrawn from ten normal animals for eight days and serum phosphatase activity was measured at four-hour intervals during the first day and daily thereafter. The results are presented in Table IV and Fig. 3.

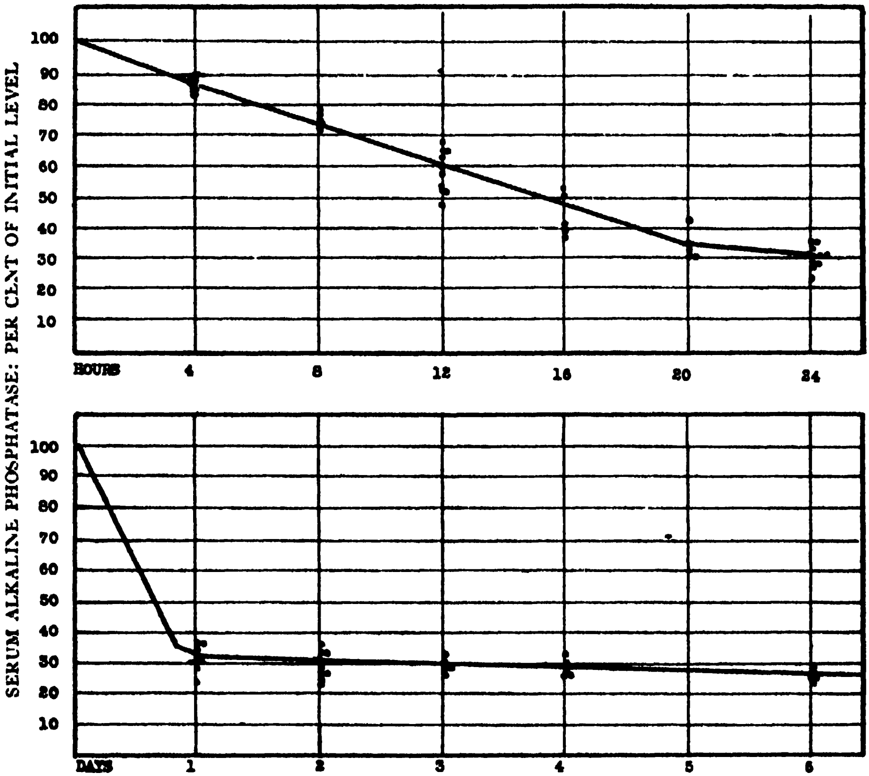


Figure 3.—Effect of fasting on serum alkaline phosphatase.

Phosphatase activity of the serum falls very rapidly during the first twelve hours of fasting, somewhat more slowly during the next twelve hours, and then levels off at about 30 per cent of normal at the end of two days and remains more or less at that level.

2. *Effect of limiting food intake*

Thirty animals were divided into five groups of six animals. The animals were housed in individual cages and given weighed quantities of food for three days. The first group received 5 gm. stock diet per animal

TABLE IV

EFFECT OF FASTING ON SERUM ALKALINE PHOSPHATASE
(units per 100 ml. serum)

Rat no.	1	2	3	4	5	6	7	8	9	10
0 hrs.	97	91	140	74	123	92	97	145	114	101
4 hrs.	84	—	116	—	104	—	84	—	97	—
8 hrs.	71	—	105	—	90	—	72	—	84	—
12 hrs.	57	55	68	47	65	58	56	100	73	53
16 hrs.	—	38	—	40	—	47	—	57	—	40
20 hrs.	—	30	—	32	—	28	—	47	—	31
24 hrs.	31	27	42	21	38	29	29	47	42	23
2 days	30	32	41	22	37	27	28	37	40	23
3 days	28	30	38	19	38	26	28	30	37	23
4 days	25	—	36	—		—	25	—	39	—
7 days	20	—	40	—	36	—	—	—	29	—
8 days	25	—	33	—	35	—	25	—	31	—

daily, the second group 10 gm. each and so on, the fifth group being given 25 gm. (Our normal animals eat between 27 and 30 gm. stock diet—Purina Fox Checkers—daily.) Phosphatase was determined at the commencement and at the end of the three-day period. The results are presented in Table V.

TABLE V

EFFECT OF LIMITING FOOD INTAKE ON SERUM ALKALINE PHOSPHATASE
(Estimations performed at the beginning (*a*) and after 3 days (*b*))

Food intake gm.		1	2	3	4	5	6
5	units *a*	133	118	120	152	84	99
	ph-ase *b*	65	72	81	71	34	39
	100 ml.						
10	" *a*	113	75	85	92	113	115
	b	56	42	50	53	69	62
15	" *a*	98	89	86	73	111	101
	b	58	68	57	54	88	71
20	" *a*	91	86	81	74	124	97
	b	79	73	68	57	112	94
25	" *a*	70	82	88	64	92	98
	b	65	68	76	60	105	95

3. *Effect of increasing food intake*

In order to obtain confirmation of the results of the previous experiment, six animals were given 5.0 gm. stock diet daily for three days. At the end of this time, the food provided was raised to 10 gm. daily for another three days and so on until they were receiving 25 gm. daily. Serum phosphatase was estimated at the beginning of the test and at the end of each three day period. These results are recorded in Table VI.

TABLE VI

EFFECT OF INCREASING FOOD INTAKE ON ALKALINE PHOSPHATASE
(5 gm. food added at 3-day intervals and estimations carried out)

	1	2	3	4	5	6
0 hrs.	133	98	106	88	129	98
after 3 days on 5 gm.	60	33	47	46	45	59
after 3 days on 10 gm.	82	49	57	55	60	60
after 3 days on 15 gm.	96	102	67	63	105	76
after 3 days on 20 gm.	112	127	79	79	113	113
after 3 days on 25 gm.	136	136	98	91	125	119

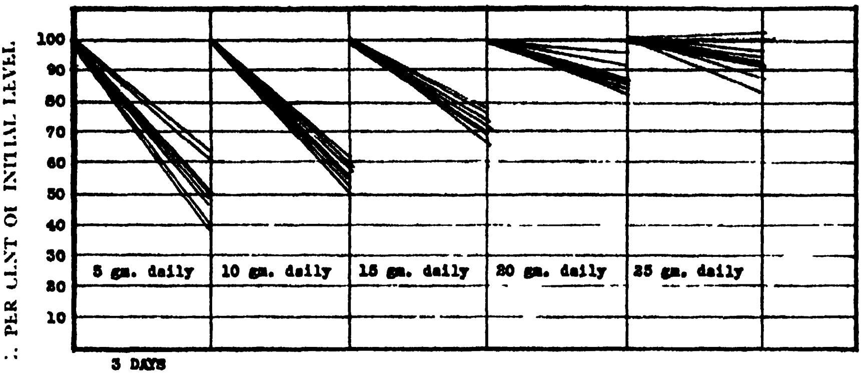
Graphic representation of the data in Tables V and VI calculated as a percentage of the initial levels is shown in Fig. 4.

It is clear from the data presented that the level of serum alkaline phosphatase increases with increased food intake. Statistical analysis of the results gave a correlation coefficient of 0.97 which is highly significant.

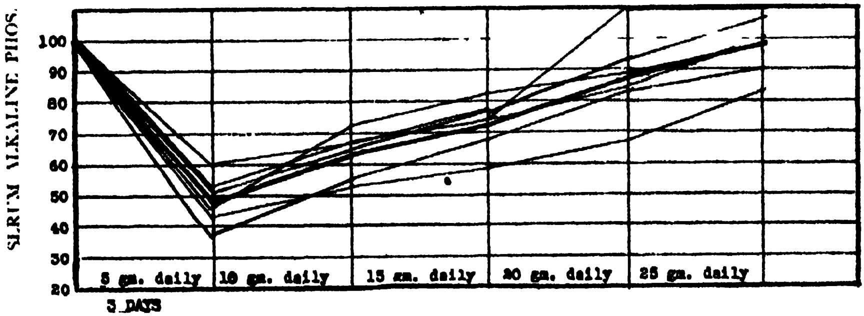
EFFECT OF COMPOSITION OF DIET ON ALKALINE PHOSPHATASE

This experiment was planned to show the effect on the phosphatase levels in normal and alloxan diabetic animals of altering the composition of the diet. High carbohydrate, high protein, and high fat diets were used. The effect of these in increasing the phosphatase activity from the low fasting was measured. Groups of six animals were used in each case. Phosphatase activity was measured before and after fasting three days, and at varying intervals for the next two or three weeks. At the end of this period the animals were provided with stock laboratory diet for a week or ten days and then re-examined. In addition, one group of six normal animals was given a diet high in carbohydrate but containing 5 per cent fat. Phosphatase estimations were carried out in this group at more frequent intervals. The results, together with the composition of the diets, are presented in Tables VII to X and are calculated as a percentage of the initial level in Fig. 5.

FIGURE 4



Effect of limiting food intake on serum alkaline phosphatase.



Effect of increasing food intake on serum alkaline phosphatase.

Examination of these data shows that the diet high in carbohydrate but containing no fat was practically ineffective in altering the low fasting level of serum phosphatase. The diet high in protein produced partial restoration of phosphatase activity, while the diet high in fat content rapidly increased phosphatase activity to the initial levels within twenty-four hours. These rose beyond the initial levels for a few days, then returned to the initial values in about two weeks. It is interesting to note that both the high protein and the high fat diets reduced the hyperglycaemia of the diabetic rats.

In the group of normal animals receiving high carbohydrate with

TABLE VII

Effect of High Carbohydrate Diet* on Blood Glucose and Serum Alkaline Phosphatase of Normal and Alloxan Diabetic Rats

Time on diet	*Control rats*		*Diabetic rats*	
	Blood glucose (mg. per cent)	Alk. phos. (units per cent)	Blood glucose (mg. per cent)	Alk. phos. (units per cent)
Before fast	188	113	460	290
After fast	88	28	135	56
1 day	160	23	500	64
2 days	145	25	450	57
3 days	112	23	530	47
4 days	96	10	590	64
7 days	110	20	430	65
10 days	130	42	500	66
14 days	150	27	450	76
18 days	130	28	500	93
21 days	—	32	540	110
7 days on normal diet	115	115	450	245

*Sucrose 75 per cent; casein 15 per cent; salt mixture 5 per cent; vitamin supplement and filler 5 per cent.

TABLE VIII

Effect of a High Protein Diet* on Blood Glucose and Serum Alkaline Phosphatases of Normal and Alloxan Diabetic Rats

Time on diet	*Control rats*		*Diabetic rats*	
	Blood glucose (mg. per cent)	Alk. phos. (units per cent)	Blood glucose (mg. per cent)	Alk. phos. (units per cent)
Before fast	100	121	500	380
After fast	65	22	125	49
6 hrs.	80	58	155	90
24 hrs.	90	40	261	58
2 days	88	54	260	164
3 days	90	56	265	154
4 days	80	65	—	—
7 days	100	—	275	152
10 days	109	51	300	132
14 days	105	60	200	161
17 days	93	55	250	147
21 days	84	56	104	—
7 days on normal diet	115	128	400	284

*Casein 88 per cent; salt mixture 4 per cent; vitamin and filler 8 per cent

TABLE IX

EFFECT OF A HIGH FAT DIET* ON BLOOD GLUCOSE AND SERUM ALKALINE PHOSPHATASE OF NORMAL AND ALLOXAN DIABETIC RATS

	Control rats		*Diabetic rats*	
Time on diet	Blood glucose (mg. per cent)	Alk. phos. (units per cent)	Blood glucose (mg. per cent)	Alk. phos. (units per cent)
Before fast	135	95	565	240
After fast	60	20	124	22
3 hrs.	70	40	140	47
6 hrs.	89	63	180	75
12 hrs.	60	97	168	138
24 hrs.	90	157	250	239
2 days	89	225	218	384
3 days	93	258	258	348
10 days	97	180	260	268
16 days	95	112	164	192
Normal diet 10 days	130	88	310	370

*Crisco 70 per cent; casein 15 per cent; salt mixture 5 per cent; cod liver oil 5 per cent.

5 per cent fat, serum phosphatase activity was restored within twelve hours. That this effect of fat might be clarified and differentiated from that described under the effect of limited food intake, an experiment was de-

TABLE X

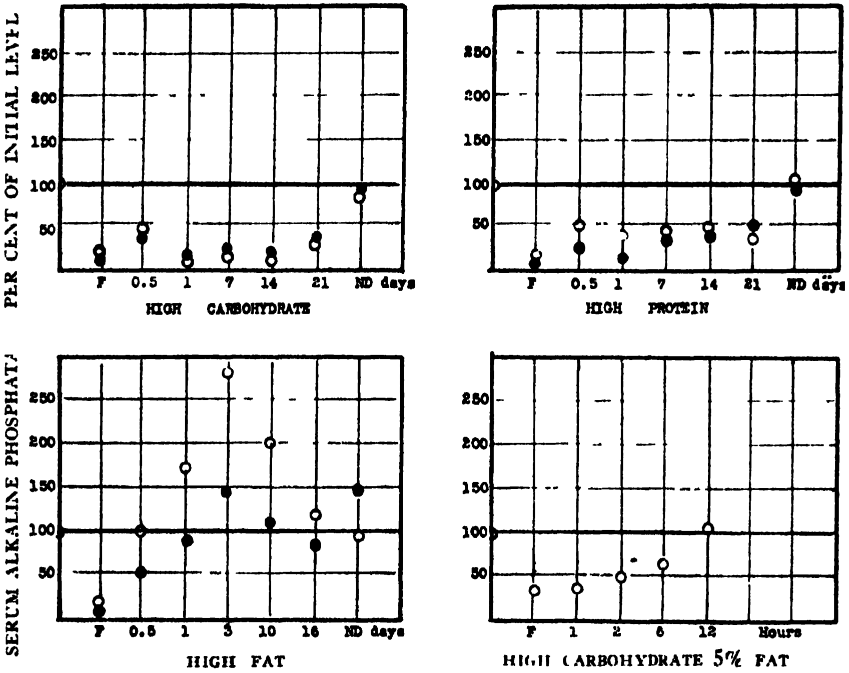
EFFECT OF HIGH CARBOHYDRATE DIET* CONTAINING 5 PER CENT FAT ON BLOOD GLUCOSE AND SERUM PHOSPHATASE OF NORMAL RATS

Time on diet	Blood glucose (mg. per cent)	Alk. phos. (units per cent)
Before fast	115	108
After "	93	45
1 hr. "	133	46
2 hrs. "	161	50
6 hrs. "	196	69
12 hrs. "	180	113
80 hrs. "	154	110
10 days "	163	109

*Starch 65 per cent; casein 18 per cent; vegetable oil 5 per cent; salt mixture 4 per cent; vitamin supplement and filler 8 per cent.

vised in which the animals were given food meeting only about 25 per cent of their normal intake.

Two groups of six animals were each given a diet providing 21 calories per day for three days. The diet of the first group contained 2 gm. fat and 1 gm. protein suitably supplemented with salt mixture and vitamins. The diet of the second group consisted of 0.37 gm. fat, 1.5 gm. protein, and 2.9 gm. carbohydrate suitably supplemented as in the first case. The results are presented in Table XI.



O Normal rats, • Alloxan diabetic rats
F—72 hours fasting; *ND*—normal stock
checker diet for 10 to 21 days.

FIGURE 5.—Effect of composition of diet on serum alkaline phosphatase of normal and alloxan diabetic rats.

From Table VI it will be seen that such animals on limited food intake show about a 50 per cent reduction in phosphatase activity. This is the case in the control group presented here numbered 1c to 6c. Examination of the data for animals 1 to 6 receiving the same calorie equivalent but with a high fat content shows practically no reduction in phosphatase activity.

TABLE XI

Effect of Fat Content of Diet on Alkaline Phosphatase
(units/100 ml. serum)

Rat No.	1	1c	2	2c	3	3c	4	4c	5	5c	6	6c
Time												
0 hr.	120	108	92	88	98	98	91	88	102	106	98	84
3 days	113	58	109	33	88	59	84	46	137	47	75	38

1-6 Rats received 21 cals./day {Fat = 2 gm.; Pr = 1 gm.}

1c-6c Control rats received 21 cals./day {Fat = .37 gm.; Pr. = 1.5 gm.; CHO = 2.9 gm.}

Discussion

Weil and Russel (1940) using normal rats who had fasted twenty-four hours observed that "only the alcohol-ether soluble fraction" of their stock diet restored the low plasma phosphatase produced by fasting. Our observations confirm this finding and extend it to include alloxan diabetic rats. Our evidence also indicates that diets high in protein have a similar but less marked effect, and that contrary to Bodansky (1933) diets high in carbohydrate have no such influence.

We have shown that although the activity of alkaline phosphatase is increased in alloxan diabetes, this increase is not due to the hyperglycaemia per se. Thus the hyperglycaemia produced by the parenteral administration of glucose or by the administration of adrenalin is attended by a decrease in phosphatase concentration. It seems possible that when glucose is not available for metabolic purposes, as in alloxan diabetes, the organism uses fat or protein. In such an instance phosphatase activity increases, in much the same manner as when fat and protein constitute the main portion of the diet.

The decreased phosphatase activity produced by insulin and by adrenalin may thus also be explained on the basis that both contribute to the utilization of glucose, and thus decrease the necessity for the conversion of fat or protein. This possibility is supported by the data represented in Fig. 2. When glucose was administered intraperitoneally to normal rats there was a reduction in phosphatase activity because these animals could use the sugar made available to them. The diabetic animals on the other hand showed no such effect because these animals could not use the injected glucose. Following the administration of adrenalin, both normal and diabetic animals responded with a reduction in phosphatase activity. In these instances the glucose enters the metabolic cycle by a different route and possibly in a different and utilizable form. It thus effects an economy of fat and protein, and phosphatase activity is reduced.

CONCLUSION

Serum alkaline phosphatase activity in normal and alloxan diabetic rats is related to the metabolism of fat and to a certain extent to the metabolism of protein. The great increase in phosphatase activity in alloxan diabetic rats occurs because the decreased utilization of carbohydrate makes it impossible for these animals to obtain the required amount of energy in the ordinary manner and therefore necessitates the decomposition of other substances such as fat and protein.

REFERENCES

BODANSKY, A. (1933). J. Biol Chem **101**: 93-104.
CANTOR, M. M., TUBA, J., and CAPSEY, P. A. (1947). Science **105**: 476-7.
GOULD, B. S. and SCHWACHMAN, H. (1943). J. Biol. Chem. **151**: 439-53.
REINECKE, R. M. (1942). J. Biol. Chem. **143**: 351-5.
SHINOWARA, G. Y., JONES, L. M., and REINHART, H. L. (1942). J. Biol. Chem. **142**: 921-33.
WEIL, L. and RUSSEL, M. A. (1940). J. Biol. Chem. **136**: 9-23.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Age and Growth Rate in the Butter Sole, *Isopsetta isolepis*

By JOHN LAWSON HART, F.R.S.C.

POPULATION constants of economically important fish species are frequently based on examinations of samples of commercial landings. The landings are frequently the results of selecting the larger fish from a more numerous catch. The catches themselves are often selected intentionally from the whole population by exploiting fishing grounds known to support the more commercially desirable individuals, or by employing selective fishing methods. Further, the catch may be selected incidently by directing fishing effort toward maturing fish which have become concentrated in restricted areas in response to breeding impulses. It seems probable that selection for any cause will lead to inaccuracy in the uncorrected estimates of the population constants when applied to the whole population. The purpose of the present discussion is to consider the nature and extent of the inaccuracies produced by applying size selection to samples of an apparently homogeneous spawning population of butter soles, *Isopsetta isolepis* Lockington, and to apply the results in establishing tentative conclusions on certain points in the life history of the species.

The butter sole is a rather small pleuronectid of good flavour. Although it has, during each of the past four years, supplied in the neighbourhood of a million pounds of fish to British Columbia trawlers the species is not highly regarded by processors because its thinness leads to excessive waste in filletting and to high labour costs. The fact that the fishery is pursued during January and February in relatively sheltered waters rather enhances its commercial importance since it offers a fishery at a time when outside fishing in northern British Columbia is very undependable.

Butter soles spawn during the latter part of February and in March. For rather more than a month prior to spawning the ripening fish accumulate in the spawning area at Skidegate Inlet on the Queen Charlotte Islands, and it is on this accumulation that the fishery depends. Non-spawning individuals are not found in the area. At the same time, butter soles which are taken in Hecate Strait are not in a spawning condition. Some males are running milt sparingly but the females all have ovaries which are either in the resting stage or are immature.

Method

The otoliths or earstones of butter soles are rather clearly marked with concentric opaque and hyaline bands resembling those usually interpreted as being annual. The left-hand (typically) otoliths from some 15,000 fish were examined on a black background, using a low power (x10) binocular microscope and reflected light. The concentric rings were counted and interpreted in years and tenths of years of age, regarding the inner edge of the hyaline band as the end of a year's growth. Most samples taken during the winter months were interpreted as being at the end of a year's growth. Of the remainder most had fractional values of .8 or .9 and the age in years was taken as corresponding to the next whole number. Fractional values of .5 and lower were disregarded in cases where they were recorded.

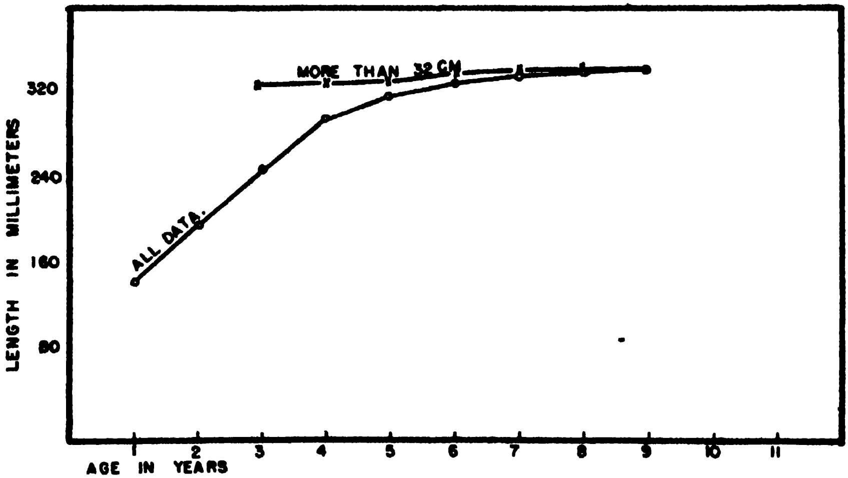


FIGURE 1.—Rates of growth of male butter soles as indicated by "unselected" and selected samples taken in Skidegate Inlet.

As a check on the reproducibility of interpretation, 1,170 of the otoliths first dealt with were re-examined. Of these 1,093 gave the same result, 54 gave lower readings and 23 higher readings on the second examination. In three cases the annulus counts differed by two.

TABLE I

Numbers and average lengths in millimetres for each group of male and female butter soles longer than specified lengths. The original samples from Skidegate Inlet, in January and February, 1944-7, unselected as to size.

	All		24 cm.		> 26 cm.		> 28 cm.		> 30 cm.		> 32 cm.		> 34 cm.		> 36 cm.	
Male	No.	Av. L.	No.	Av. L.	No.	Av. L	No	Av. L.	No	Av. L.	No.	Av. L	No.	Av. L.	No.	Av. L.
Age																
II	11	145														
III	131	205	10	261	4	272										
IV	315	255	199	274	121	286	48	304	17	321	4	335	1	350		
V	358	295	327	301	286	307	239	314	143	326	67	338	13	359	3	373
VI	334	318	327	319	308	323	286	327	212	333	158	342	52	356	7	374
VII	267	335	267	335	259	338	247	341	241	342	205	347	113	356	19	374
VIII	99	343	99	343	97	345	82	346	94	347	84	350	54	358	12	375
IX	33	346	33	346	33	346	33	346	32	347	31	348	18	357	2	385
X	4	352	4	352	4	352	4	352	4	352	4	352	2	365	1	370
Female																
Age																
II	1	260	1	260												
III	10	226	3	253												
IV	30	294	26	305	23	312	20	317	13	330	9	334	1	350		
V	169	344	168	345	168	345	167	345	164	346	142	351	91	359	20	377
VI	257	357	257	357	256	257	256	357	253	358	245	359	192	365	89	377
VII	225	366	225	366	225	366	225	366	225	366	223	366	205	369	117	380
VIII	76	376	76	376	76	376	76	376	76	376	76	376	73	378	55	384
IX	59	385	59	385	59	385	59	385	59	385	59	385	59	385	32	386
X	7	394	7	394	7	394	7	394	7	394	7	394	7	394	7	394

Discussions of Results

The results of the annulus counts were assembled according to the locality of the fishing ground, the season of the year, whether or not the fish had been deliberately selected according to size before sampling, and sex. Each group was then broken down into two-centimetre length classes, and the number of fish of each age and the average lengths for each age tabulated for all the fish in and larger than each size category. The procedure will be more evident upon examining composite Table I. The effect of selection upon estimates of age distribution and average sizes at each age among the males is very evident. In Fig. 1 are shown growth curves for unselected data and data selected at the 32 cm. level. The effects of selection on the essential estimates for females are negligible. This is because the population of females although unselected by man is composed entirely of sexually mature individuals with the result that the smaller and younger females are excluded. This interpretation is supported by examining contracted Table II based upon unselected landings of non-spawning fish taken during the spawning season.

TABLE II

Numbers and average lengths in millimetres for groups of male and female butter soles longer than specified lengths. The original samples from Butterworth Rocks fishing ground in north-eastern Hecate Strait, February and March, 1945 and 1947, unselected as to size.

Age	Males						Females					
	All		> 30 cm.		> 32 cm.		All		> 30 cm.		> 32 cm.	
	No.	Av. l.	No.	Av. l.	No.	Av. l.	No.	Av. l.	No.	Av. l.	No.	Av. l.
II	8	139					3	167				
III	32	232					55	239				
IV	147	275	5	310			287	283	38	315	4	337
V	21	287	4	330	1	360	50	298	21	319	5	340
VI	3	287	1	310			7	306	3	333	2	345
VII							1	340	1	340		

Comparison of the average lengths for each age in the "all" column of Tables I and II suggests especially for males, but to some extent also for females, that sexual precocity is deterrent to growth but that sexual inactivity among older fish is associated with retarded growth.

The average sizes of butter soles as estimated from all unselected data are given in Table III. Because some selection still exists, these

TABLE III

Average lengths of male and female butter soles at each age

	Average length millimetres			Average length millimetres	
Age	*Male*	*Female*	*Age*	*Male*	*Female*
II	143	190	VII	335	366
III	210	237	VIII	343	376
IV	261	284	IX	346	385
V	294	334	X	352	394
VI	318	355			

estimates are high for the lower age groups. The greater lengths of females at all ages will be noted.

Culling as carried out commercially does not employ a rigid standard of selection. Small fish are accordingly landed and can be used to reach erroneous conclusions. The possibility for error is shown by comparing the average lengths and age distributions from commercial catches as shown in Table IV with the corresponding values in Table I. In most comparisons the values determined for samples of selected fish are higher. In other cases the differences are not believed to depend upon selective or biological factors.

TABLE IV

Numbers and average lengths in millimetres of groups of male and female butter soles longer than specified lengths. The original samples from Skidegate Inlet, January and February, 1945-8, culled commercially.

Age	MALES						FEMALES					
	All		> 30 cm.		> 32 cm.		All		> 30 cm.		> 32 cm.	
	No.	Av. l.	No.	Av. l.	No.	Av. l.	No.	Av. l.	No.	Av. l.	No.	Av. l.
III	6	230					1	280				
IV	113	291	32	317	3	340	72	322	54	332	32	343
V	758	317	556	325	249	338	792	341	766	347	648	348
VI	718	331	655	334	456	342	768	355	759	355	726	357
VII	449	339	433	341	353	346	477	367	477	367	467	367
VIII	135	344	129	346	115	349	174	373	172	374	171	374
IX	17	349	17	349	16	351	34	379	34	379	34	379
X	2	360	2	360	2	360	17	383	17	383	17	383

A fishery for butter soles has developed around June off Fife Point. Table V shows the numbers and lengths for different ages and degrees of selection. The differences in averages between Tables IV and V are due (1) to the period of growth between the spawning season and June, and (2) the method of "rounding" results to the nearest whole year.

A small sample taken in Hecate Strait in June, 1945, showed generally lower lengths than Fife Point samples in corresponding categories. Variations in culling practice may account for the observation, but biological explanations are not ruled out.

TABLE V

Numbers and average lengths in millimetres of groups of male and female butter soles longer than specified lengths. The original samples from Hecate Strait off Fife Point, June, 1945 and 1946, culled commercially

Age	Males						Females					
	All		> 30 cm.		> 32 cm		All		> 30 cm		> 32 cm.	
	No	Av. l	No	Av. l	No.	Av. l	No	Av. l	No.	Av. l	No	Av. l
II	1	140					3	200				
III	7	233					20	263	5	324	2	340
IV	46	290	10	317	3	333	118	304	59	324	27	338
V	97	315	64	330	34	342	186	326	149	334	96	344
VI	116	338	108	342	87	348	118	354	115	356	107	359
VII	156	350	153	352	144	354	118	368	118	368	113	370
VIII	86	356	86	356	86	356	71	380	71	380	71	380
IX	29	357	28	358	28	358	33	388	33	388	33	388
X	2.	345	2	345	2	345	18	393	18	393	18	393

From the foregoing tables the effect of selection on age distribution in samples is apparent and the reasons for the effect obvious. Selection distorts the age distribution toward older ages. In Table VI is given the age distribution from all winter samples by sex and year. There is no strong evidence for believing either that year class strength is constant or that dominance in year classes is characteristic of the butter sole population. No pattern exists which is strong enough to show through the effects of selection and the vicissitudes of the fishery. The decline in the average age composition appears to be real. In 1944, the best represented ages were six and seven. In 1945, seven and six had stronger representation. In 1946, six, five, and seven were predominant. In 1947 and 1948, five and six were most prevalent. There would appear to be a regular trend toward younger fish in the catches and landings.

TABLE VI

Age distribution of butter soles taken in the winter months in Northern British Columbia waters, by season and sex

Age		10	9	8	7	6	5	4	3	2
1944	♂	—	3	15	26	44	27	25	15	1
	♀	1	3	5	23	18	5	5	6	1
1945	♂	4	13	40	124	129	80	143	135	18
	♀	12	13	42	128	102	47	64	43	2
1946	♂	1	24	125	333	319	291	161	13	—
	♀	8	28	106	318	348	323	303	57	—
1947	♂	1	10	48	199	395	502	316	14	—
	♀	3	23	80	183	378	542	73	3	—
1948	♂	—	—	7	37	173	281	14	—	—
	♀	-	2	18	55	222	366	23	—	—

Such a change is an expected accompaniment of a developing fishery.

When mortality rates are calculated from age distributions the estimates are affected by selecting only the larger fish, but not to a very great extent, as the calculations are based on the older individuals in any case. In the case of the butter soles evidence of constant total mortality rate has not been obtained. This is probably due to the short life of the fishery but may be the result of biological factors. Mortality rates for annual intervals are accordingly presented for comparison, the mortality rate between ages nine and ten being calculated thus,

$$\frac{100\ (\text{number of nine year olds} - \text{number of ten year olds})}{\text{number of nine year olds.}}$$

TABLE VII

Percentage mortality rates in male and female butter soles of each sex as indicated for different age intervals by commercial and unselected samples

Age interval *Years*	Males		Females	
	Unselected	*Selected*	*Unselected*	*Selected*
7– 8	63	70	48	63
8– 9	67	80	52	81
9–10	88	93	82	50

The results are shown in Table VII. Male mortality rates appear to be higher than those for females. For males, rates based on unselected samples are higher than those based on commercial samples. The effects of selection on estimates of mortality rates for females are not clear.

In recapitulation: Selection, deliberate or incidental, of butter soles for length, modifies estimates of growth rate and age distribution rather radically although less so among older fish. Average sizes for each age and sex are given but must be regarded only as approximations. There is no evidence of dominance of year classes but a decline in age composition with the life of the fishery is indicated. Estimates of mortality rates do not indicate them to be stabilized. They are affected by selection and are greater for males than females.

The results are based on otolith collections made by several employees of the Pacific Biological Station. Otolith readings and many of the calculations were made by Miss Winona Bethune to whom I am very grateful.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Plant Growth in relation to the Bacterial Equilibrium in Soil[1]

By A. G. LOCHHEAD, F.R.S.C.

INTRODUCTION

In soil there is increasing evidence of the existence of a microbiological equilibrium, or more accurately stated, the constant striving towards an equilibrium, for it is ever changing under the influences of season, temperature, moisture, treatment, and cropping system. Conditions in the soil are not analogous to those prevailing, for instance, in an infected animal or in contaminated milk or food. Such conditions in the latter are "abnormal"; it is easier to recognize cause and effect, and there are fewer microbial associations and antagonisms. Thus, whereas the ideal state of animal or plant tissue, potable water, and most foodstuffs implies relative freedom from microbial activity, that of the soil connotes abundance of micro-organisms. Hence in soil, microbes are indigenous in a sense not true of most other media; and the soil microbiologist is forced to consider, not only an abundance of organisms, but associations and antagonisms of different types as normal conditions in the medium he is studying. The present paper is concerned with certain aspects of the bacterial balance, particularly as it is affected by plant growth. In the course of the studies a number of associates have collaborated, and their co-operation in various aspects of the work is acknowledged.

EXPERIMENTAL

A study of the microbial balance in soil implies the use of qualitative procedures. Previous methods for the qualitative study of soil bacteria have involved the use of highly selective media, for plate or dilution counts, chosen to favour definite physiological groups. The approach developed in this laboratory, on the other hand, requires the use of as non-selective a medium as possible for pure culture study. By following systematic isolation methods the procedure is also made quantitative and permits the determination of the percentage incidence of various types of organisms.

---

[1] Contribution no. 259 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

As the least selective medium available, soil extract agar, without added energy material, is used for plating. From representative plates, after twelve to fourteen days' incubation at 26° C., colonies are systematically picked so that all on a plate or sector are taken and stab inoculations made into soil extract semi-solid (0.02 per cent K_2HPO_4, 0.01 per cent yeast extract, 0.5 per cent agar) for further study.

MORPHOLOGY AND GENERAL PHYSIOLOGY

In soil of a definite type, apart from the influence of plant roots, there exists a surprisingly uniform balance between the various morphological and physiological groups of bacteria, even though the productivity may be altered greatly by manurial treatment. This is illustrated in Table I.

TABLE I

RELATIVE INCIDENCE OF MORPHOLOGICAL AND PHYSIOLOGICAL GROUPS OF BACTERIA IN SOILS OF DIFFERENT FERTILITY

	Soil X (no fertilizer 25 years)	*Soil X (farm-yard manure)*	*Soil Y (mineral fertilizer)*
Relative crop producing capacity			
Timothy	64.3	100.0	81.7
Mangels	9.4	100.0	91.7
Oats	72.5	100.0	90.3
Clover	41.9	100.0	77.5
Morphological groups	Per cent	Per cent	Per cent
Short rods, gram-neg.	30.5	35.2	42.1
Short rods, gram-pos.	29.6	30.5	24.5
Short rods, gram-var.	7.0	10.8	10.3
Bact. globiforme group	11.2	7.5	8.3
Coccoid rods, gram-pos.	8.4	5.6	3.9
Cocci	5.6	3.3	2.4
Long rods, non-sporing	3.3	3.8	4.4
Spore-formers	4.2	3.2	3.9
Some physiological groups	Per cent	Per cent	Per cent
Little or no growth—nutrient agar	53.5	58.7	66.1
Gelatin liquefaction	28.0	27.7	29.4
Nitrate reduction	40.4	43.7	30.4
Acid production (glucose)	42.7	38.5	43.6

Striking differences in fertility have resulted from different treatments over a twenty-five year period; yet the character of the bacterial flora has remained relatively uniform.

In proximity to the growing plant, however, the bacterial equilibrium in the soil is altered qualitatively, as is shown by definite changes in the relative incidence of different bacterial groups. Apart from the increase in total numbers known to occur in soil adjacent to the roots, there is evidence of a selective action characteristic of the rhizosphere of all plants studied. Of the various groups referred to in Table I, four are noticeably affected by plant growth. As shown in Table II, gram-negative short rods are preferentially increased while gram-positive short rods, coccoid rods, and spore formers are relatively suppressed.

TABLE II

"RHIZOSPHERE EFFECT" ON THE INCIDENCE OF CERTAIN MORPHOLOGICAL GROUPS OF SOIL BACTERIA

	Short rods, gram-neg.		*Short rods, gram-pos.*		*Coccoid rods*		*Spore formers*	
	C	Rh	C	Rh	C	Rh	C	Rh
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Red clover...	30.3	55.1	19.7	8.7	13.6	5.8	6.1	2.9
Mangels...		43 1		10.8		10.8		0.0
Oats.......		45.5		13.6		10 6		3 0
Tobacco I	13.3	43.3	23.3	20.0	18.3	10.0	10 0	1.7
Tobacco II ..		45.2		16 1		4.8		0.0
Corn....	26.9	51.1	23.1	22.2	21 2	13.3	9.6	0.1
Flax I....	15.4	36.4	7.7	6.1	53.8	32 3	5 8	1.0
Flax II ...		59.1		1.4		16.9		0.0
Flax I......	15.8	40.2	5.3	3.1	50.5	43.3	7.4	0.0
Flax II.......		40.0		3.2		41.1		0 0

C = control soil.
Rh = rhizosphere soil.

One of the outstanding effects of plant growth is the stimulation of the activity of the micro-organisms. In the rhizosphere there is an increased proportion of proteolytic and saccharolytic, of chromogenic and motile forms, which considered in conjunction with the numerical increase, points to one of the outstanding influences of plant growth on the microbial balance in soil (Table III).

TABLE III

"RHIZOSPHERE EFFECT" ON THE INCIDENCE OF SOME PHYSIOLOGICAL GROUPS OF SOIL BACTERIA

	Good gr. on nutr. agar	*Chromogenic*	*Motile*	*Gelatin liq.*	*Acid reaction dextrose*	*Alkaline reaction dextrose*
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Control soil..........	33.3	12.1	--	—	39.4	6.1
Rhizosphere—clover.....	56.5	29.0	—	—	42.1	15.9
Rhizosphere—mangels.	61.5	29.2	—	--	53.8	16.9
Rhizosphere—oats.....	45.5	21.2	—	—	43.9	21.2
Control soil...........	35.0	1.7	13.3	15.0	36.7	16.7
Rhizosphere—tobacco I .	75.0	26.7	31.7	16.7	38.3	21.7
Rhizosphere—tobacco II	66.1	29.0	25.8	41.9	48.4	17.7
Control soil..........	48.1	3.8	13.5	23.1	56.7	9.6
Rhizosphere—corn.	57.7	33.3	24.4	24.4	57.8	17.8
Control soil..........	36.5	7.7	26.9	42.3	50.0	3.9
Rhizosphere—flax I...	78.8	20.3	36.4	54.6	67.7	9.1
Rhizosphere—flax II ...	85.9	25.4	64.8	70.4	81.7	12.7

THE "NUTRITIONAL" APPROACH TO THE STUDY OF THE BACTERIAL EQUILIBRIUM IN SOIL

In assessing the significance of changes of equilibrium, it is felt that other criteria than the taxonomic classification based on morphology or general physiology are more suited to the grouping of bacteria, if this is to contribute towards a better understanding of the relationship of soil micro-organisms to plant growth and health.

Since the equilibrium between various groups of organisms in soil will depend in large measure upon the availability of nutrients required for growth, a method has been developed for classifying soil bacteria according to their nutritional needs. The method involves the determination of the growth response of organisms in seven cultural media, isolated on a non-selective basis by the procedure referred to. These range from a simple basal medium to those of increasing complexity, containing respectively amino acids, growth factors, amino acids plus

TABLE IV

Classification of Soil Bacteria according to Nutritional Requirements

Differential media		*Nutritional group*
B	Basal medium (glucose-salts)	I
A	B + amino acids	II
G	B + growth factors	III
AG	B + amino acids + growth factors	IV
Y	B + yeast extract	V
S	B + soil extract	VI
YS	B + yeast extract + soil extract	VII

growth factors, or the unidentified substances present in yeast or soil extract, or both. Depending upon the medium providing maximum growth, the organisms may be divided into seven main "nutritional groups" (Table IV).

Studies of the "rhizosphere effect" on the balance between different nutritional groups indicate that the growing plant exerts profound, and apparently characteristic, changes in the equilibrium. First observed with mangels, these have since been noted with a variety of plants, as shown in Tables V and VI. The outstanding features of the rhizosphere

TABLE V

The "Rhizosphere Effect" in relation to the Nutritional Requirements of Bacteria

	Mangels (1945)		*Wheat* (1947)		*Oats* (1947)	
	C	Rh	C	Rh	C	Rh
Plate count (millions/gm.)	37	532	123	701	250	2,660
Nutritional group	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
I. Basal medium.....	12.0	22.5	9.3	36.7	12.0	33.1
II. Amino acids.......	6.8	25.0	3.6	5.6	2.5	11.0
III. Growth factors.....	23.1	15.0	11.4	20.0	8.4	11.0
IV. Amino a. + gr. fact.	16.2	15.0	0.7	2.8	1.4	0.0
V. Yeast extract......	16.2	11.7	18.6	19.0	25.4	19.3
VI. Soil extract.... ...	6.8	5.8	27.8	11.7	21.8	17.3
VII. Yeast ex. + soil ex.	11.1	2.5	17.8	3.3	23.2	7.6

C = control soil.
Rh = rhizosphere soil.

effect are (1) the preferential stimulation of organisms with simple requirements and of those responding to amino acids, and (2) the relative suppression of bacteria dependent upon the nutrients provided in soil extract. It is therefore the balance between Groups I and II on the one hand, and VI and VII on the other (particularly VII) which is normally influenced by the growth of plants.

The shifting of the equilibrium resulting in a preferential stimulation of bacteria requiring amino acids has been observed, in addition to the crops mentioned in Tables V and VI, also with tobacco, potatoes, strawberries, and tomatoes. The increased incidence of this group of organisms is of interest in relation to plant excretions, the nature of which is still quite obscure. Though the findings do not provide direct evidence of the excretion of amino acids, they are consistent with the view that such products may be excreted in amounts sufficient to modify the bacterial balance in the region of the plant root. The formation of amino acids as decomposition products of sloughed-off portions of roots is also a possible, though not the sole factor, since the stimulation of bacteria requiring amino acids may be detected in the case of seedling plants.

TABLE VI

THE "RHIZOSPHERE EFFECT" IN RELATION TO THE NUTRITIONAL REQUIREMENTS OF BACTERIA

	Timothy, alfalfa, red clover (1947)				*Flax* (1947)	
	C	Timothy Rh	Alfalfa Rh	Clover Rh	C	Rh
Plate count (millions/gm.)	133	826	830	2,650	119	1,119
Nutritional group	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
I. Basal medium.....	5.4	20.5	27.5	34.2	7.7	26.0
II. Amino acids .	2.3	5.4	11.0	20.5	2.6	9.8
III. Growth factors ..	10.0	11.4	14.4	12.9	9.0	12.7
IV. Amino a. + gr. fact.	10.0	8.1	10.0	9.0	8.4	5.2
V. Yeast extract......	17.7	13.5	14.9	11.0	11.0	30.0
VI. Soil extract..... ..	33.7	28.6	14.9	7.7	24.5	12.1
VII. Yeast ex. + soil ex.	11.5	9.2	3.8	3.3	36.8	4.0

C = control soil.
Rh = rhizosphere soil.

ASSOCIATIONS AND ANTAGONISTIC EFFECTS

Though the equilibrium between various groups of organisms in a soil will depend largely on the availability of special nutrients required by these organisms, associative and antagonistic effects may well play a part in establishing the microbial balance.

To note whether such effects could be detected, a study was made of the action of Group I organisms on those of Groups II and III respectively. The effect of sterile culture filtrates of Group I organisms, grown in the basal medium (B), was observed on the growth of the other groups (1) in the basal medium, in which they normally do not grow, and (2) in media A and G, respectively, in which they grow well. The results, shown in Table VII, indicate that as far as the organisms tested are concerned, bacteria of Group I stimulate those of Group II more than Group III and, on the other hand, are more antagonistic towards Group III. The tests, though limited in scope, provide evidence to support the idea that in the establishment of a microbial equilibrium, associative and antagonistic effects are to be considered.

TABLE VII

ASSOCIATIVE AND ANTAGONISTIC EFFECTS OF GROUP I BACTERIA ON GROUPS II AND III

	Group II bacteria (amino acids)		*Group III bacteria* (growth factors)	
	Number	Per cent	Number	Per cent
Number of tests made on effect of filtrates of Group I bacteria	90	100 0	88	100.0
Grow in basal medium	0	0.0	0	0 0
Grow in basal medium on addition of filtrates of Group I bacteria	42	46.2	16	18.2
Grow in Medium A	90		—	
Grow in Medium G	—		88	
Suppressed in Medium A (or G) on addition of filtrates of Group I bacteria—partially	4	4.4	24	27.3
completely	0	0.0	10	11.4
Total	4	4.4	34	38 7

Studies of populations, whether concerned with animal, plant, or microbial ecology, have all suggested a continuous striving towards an equilibrium in any environment. In soil the micro-population may likewise be said to be in a state of dynamic equilibrium. In arable soils it is suggested that the main factor affecting the balance between bacterial types is the growing plant. Though changes in the bacterial equilibrium may be detected among morphological and certain general physiological groups, it is considered that an approach to the problem, capable of providing more knowledge of the meaning of the changes in balance, lies in a study of micro-organisms from the standpoint of their nutritional requirements.

Methods suggested in these studies have proved helpful in certain directions and are being adapted to investigations of soil-borne plant diseases. Doubtless, details of procedure will be modified and improved upon, but it is felt that the development of the principles concerned will provide a useful tool for qualitative investigations of the complex microflora of the soil and its relationship to important problems of crop production.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Preliminary Studies on Muskrat Biology in Manitoba

By J. A. McLEOD

Presented by W. LEACH, F.R.S.C.

LOW-LYING plains, some of which extend for upwards of one million acres, are moderately abundant in the Hudson Bay drainage area, particularly along larger watercourses or in the vicinity of larger prairie lakes. Under rare conditions of extreme drought many of these plains temporarily become dry enough for agricultural purposes, but under conditions of normal or heavy precipitation they are much too wet even for grazing purposes. Thus, their greatest value appears to lie in their utilization as habitats for water-frequenting birds and fur-bearing mammals.

From the point of view of revenue derived, fur-bearing mammals are of greater importance than birds, and among the various species concerned the North American muskrat (*Ondatra zibethica*) is preeminent. Because of this rodent's fine fur quality, high fecundity, and adaptation to marsh life, many areas of otherwise low productivity have come to yield a considerable yearly revenue.

In the great majority of marsh areas strict regulations have been adopted governing the time of trapping and the size of the pelt crop harvested in order to ensure the survival of an adequate breeding population. In a few cases water control measures have been undertaken in order to increase or maintain the supporting capacity of the habitat. However, in spite of these measures, the sustained high production desired has not always been achieved, but rather it was found that while the results were often gratifying they were extremely unpredictable and occasionally disappointing. The disturbing feature was that it was impossible to explain why enormous variations should occur in an area where environmental conditions appear to be reasonably stable from year to year.

The existing literature contains a good deal of valuable information on the feeding and breeding habits of the muskrat in other parts of North America, and on the roles played by drought, disease, and predation as parts of the environmental resistance in limiting muskrat populations.

Much of this information is applicable to Manitoba problems, but in the absence of drought and any reports of extensive predation or disease it was felt that the entire situation should be investigated.

The following report is based on preliminary studies carried out briefly in 1944 and 1945, and during the summer of 1947, by the writer and Mr. D. R. Moir, Provincial Biologist, with the aid of two student assistants, all working under the auspices of the Department of Mines and Natural Resources, Province of Manitoba.

For purposes of study the problem of muskrat management was broken down into two phases, namely, a study of the biology of the muskrat itself and a study of the supporting capacities of local habitats. In the main the problem is concerned with marsh muskrats. Although burrow-inhabiting individuals have not been ignored they are relatively so rare as to be unimportant.

SOIL AND WATER

The area under investigation was a large flood plain involving approximately a million acres bordering on the Saskatchewan River and its branches. The parental soil type of the area is Silurian limestone (Ellis, 1938) over most of which lies a layer of gravel of variable thickness from the bottom of the now extinct Lake Agassiz. It is surprisingly flat with an incline to the northwest of only about twenty feet over a distance of forty-seven miles. It appears that as Lake Agassiz receded the inflowing river tended to build up a plain of clay and silt and a delta was formed. Recession of the lake was accompanied by an extension and broadening of the delta as the water currents decreased and the heavy suspension settled out.

The Saskatchewan River in Manitoba shows two flood periods per year, the first representing the local spring run-off with the peak occurring on the average somewhere between April 18 and May 6, and with an average rise over the past ten years of twelve feet six inches. The second flood period normally occurs sometime between June 12 and August 9, representing the run-off from melting snows in the Rocky Mountains and showing an average rise of ten feet five inches.

At the present time under peak flood conditions, the Saskatchewan River spills over its banks in many places in the delta region and fills the numerous depressions with water. There is a tendency toward dyke formation along the banks of channels and also some silting-in of channels and depressions where currents are reduced (Fig. 1). The result of such water action has been the building up of an enormous, very wet plain in the delta region, the water depth of the extensive depressions rarely exceeding eight or ten feet. Outcroppings of the original limestone or the gravel are quite small and rather rare. The great bulk of the soil has been borne in and deposited by water from the Rocky Mountains and the western plains. Some thin strata of sandy silt were found in localized spots (Fig. 2), but much of the soil is a heavy, dark grey, gumbo clay which is quite sticky when wet but bakes very hard and can be reduced to a fine powder when dry. The water capacity of this soil is reasonably high and, as seen under the microscope, the particles are small with little or no intermingled fibre. Thus, the flood plain of the delta region appears to represent an island of black earth alluvial deposit in an extensive area where the soil generally is of the Rendzina type.

On higher land where flooding is less common or less prolonged, a moderate layer of humus has been built up, but in the marshes and meadows proper humus layers are thin and, if present at all, are buried frequently under successive layers of clay. Vegetable matter so buried appears to undergo little if any decomposition, undoubtedly due to the lack of oxygen and the very wet condition. Stalks and leaves of *Phragmites maximus* and other marsh plants which had obviously been buried for some time were found in a good state of preservation under successive layers of deposit a foot in thickness.

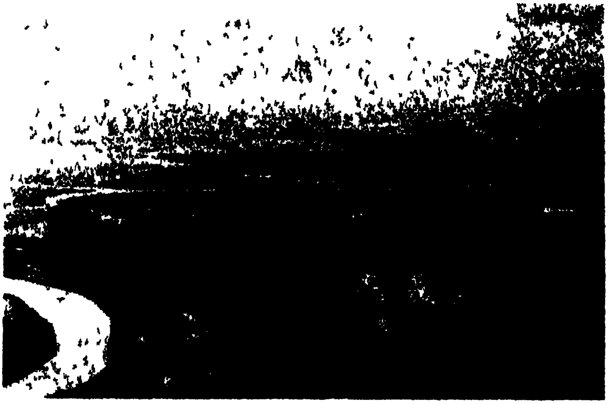


FIGURE 1.—Aerial view of portion of flood plain showing channels and sloughs

The soil and water were alkaline and when subjected to analysis showed the presence of carbonates and bicarbonates of calcium and magnesium of from 7.3 p.p.m. in some recently flooded areas to a maximum of 151 p.p.m. in areas which had been flooded for ten years or more. The hydrogen ion concentration of marsh water varied considerably from place to place and from time to time. The entire range encountered was from pH 6.7 to pH 8.1. While negative and positive ions are undoubtedly produced in considerable quantity, it is to be borne in mind that a good many buffers are present in both the soil and the water. Undoubtedly due to the liberation of organic acids the pH was lowest where the water was most stagnant and the greatest amount of partial decomposition of organic material was taking place. Generally speaking, shallow water in dense vegetation was slightly acid just after the disappearance of the ice in the spring. As one proceeded from shallow to deep water it became basic. Surface water became progressively more basic as the summer advanced, irrespective of the type of vegetation in the vicinity, but bottom water showed a sharp incline to the basic side until the end of the season of maximum plant growth, and then became progressively less basic.

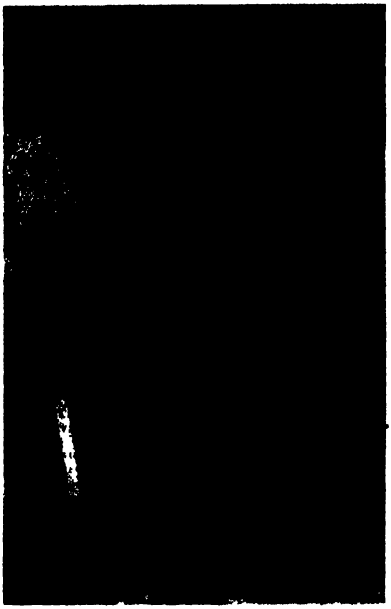


FIGURE 2.—Side of pit dug near river channel showing stratification of silt and clay.

The oxygen concentration of the marsh water was found to vary from 2 p.p.m. to 10 p.p.m. depending on the interval since the disappearance of ice, the opportunity for aeration, and the presence or absence of decomposing vegetation. The lowest concentration occurred, of course, in spring in shallow water with dense vegetation. In terms of percentage saturation, bottom samples showed about ten per cent less oxygen than surface samples. The increase in oxygen concentration kept pace with the increase in the rate of growth of the plants and reached a maximum in mid-July following which there was a gradual decline.

In many cases the bottoms of ponds and sloughs were found to be moderately firm and gases were absent. In other cases where quantities of vegetation had fallen into the water on several successive years, complete decomposition had not taken place, and the clay was overlain by a layer of black, coarsely flocculent debris. It was extremely soft, contained an abundance of methane, and frequently had a strong odour of sulphides, etc. The water was exceedingly turbid in most cases until about July 1, when the fine clay and silt had largely settled out.

VEGETATION

The Saskatchewan River delta lies in the Manitoba lowlands section of the boreal forest region (Halliday, 1937) which has a rather characteristic vegetation. Generally speaking the forest cover is of black and white poplar mixed with white spruce in the better drained portions, and with tamarack and black spruce in the poorly drained situations. Restricted patches of jack pine, oak, and cedar are found. The delta is atypical because of its different soil nature and poor drainage, except where higher limestone outcroppings occur. Here small bluffs of black spruce and some poplar are found. The dykes along the banks of channels and high black earth points are characterized by a dense growth of willows of various species, most of which are in the form of tangled masses due to snow and ice action.

The greater part of the area has reached the marsh and meadow stages of ecological evolution which may be regarded as early land seres. The underlying causes of the difference between the delta and the balance of the Manitoba lowlands section are, of course, the presence of black earth several feet in thickness and also the water abundance.

The following, while not a complete floral list, includes the forms which comprise the bulk of the plants of the marsh and meadow portions of the delta region:

- Typhaceae
    - *Typha latifolia*
- Graminaceae
    - *Phragmites maximus*
    - *Glyceria grandis*
    - *Agropyron pauciflorum*
    - *Agrostis alba*
- Cyperaceae
    - *Scirpus fluviatilis*

- *Scirpus validus*
- *Carex rostrata*
- *Carex lacustris*
- *Eleocharis palustris major*

Equisetaceae
- *Equisetum fluviatile*
- *Equisetum arvense*

Alismaceae
- *Alisma plantago-aquatica*
- *Sagittaria cuneata*
- *Sagittaria latifolia* (variations)

Araceae
- *Acorus calamus*

Compositae
- *Bidens* (sp.?)
- *Megalodonta beckii*

Haloragidaceae
- *Hippuris vulgaris*
- *Myriophyllum alterniflorum*
- *Myriophyllum heterophyllum*

Labiatae
- *Mentha canadensis*

Lentibulariaceae
- *Utricularia vulgaris* (?)

Polygonaceae
- *Polygonium amphibian*

Scheuchzeriaceae
- *Triglochin palustris*
- *Triglochin maritima*

Sparganicaceae
- *Sparganium eurycarpum*

Umbelliferae
- *Sium* (sp.?)
- *Sium cicutaefolium*

Vallisnereaceae
- *Elodea canadensis*
- *Vallisneria* (*sp.?*)

Lemnaceae
- *Lemna sp.*

Of these *Agropyron pauciflorum, Agrostis alba, Carex rostrata, Carex lacustris*, and *Alisma plantago-aquatica* are the predominants of the meadow or somewhat drier portion, while *Phragmites maximus, Typha latifolia, Scirpus validus*, and *Equisetum fluviatile* are the predominants of the marsh or emergent plant zone.

The study of the responses of the plants to flood conditions was greatly facilitated by the fact that a portion of the delta area had been under flood for ten years while other portions had been under flood for various lesser periods ranging down to one year. It was observed that shortly after flooding there is a general drowning out of the existing vegetation and a recession of all emergent plants toward shallower water or drier soil. Thus, for about two years, the usual marginal zonation of plants is somewhat confused and young marsh plants are found establishing themselves amongst meadow forms which are in the process of dying out and becoming re-established beyond the water's edge. Providing conditions remain reasonably stable, and particularly if the water level does not rise, zonation of plants again becomes apparent and a maximum growth is obtained in four or five years following flooding. It was found that emergent forms normally did not occur in water in excess of thirty inches in depth and that signs of drowning appeared when the water depth exceeded two feet. From the inner fringe of emergent vegetation outward toward higher ground the commonest order of the plant species was as follows:

FIGURE 3—Marginal vegetation taken from the shore side looking out toward deeper water, showing *Phragmites* in foreground and *Typha* in the distance

Scirpus and *Equisetum*
Typha
Phragmites
Sagittaria
Agropyron, *Agrostis*, *Carex*, *Alisma*, etc.

Usually the *Phragmites* zone is relatively broad and extends from above the water's edge out to a depth of one foot or eighteen inches (Fig. 3). If the water level was allowed to rise appreciably after the plants had become established, uprooted masses of plants of various species occurred along the outer margin as a result of what appeared to be drowning and wave action.

MUSKRATS

Because of the very level nature of the terrain and the high water of the delta region, very limited areas are available for burrowing where muskrats are able to make nests above the high water mark. Some of the channels with high marginal dykes are suitable for this purpose but their area is relatively small. Continued observations have led to the assumption that the great percentage of muskrats inhabiting the delta are house-dwelling forms, and within the larger general habitat it is apparent that the muskrat has a very specific habitat which includes only the marginal strip of vegetation around each lake or slough from the water's edge out to open water. Here the animal makes its house and must find most of its food and coverage. Thus, the supporting capacity of the marsh is dependent on the size of the marginal strip of emergent plants and on their quality and quantity.

Large molluscs, crayfish, and larger insect naiads are practically absent from the area so the muskrat is restricted largely to a vegetable diet. In summer, from June 10 to September 15, fresh stalks, shoots, and leaves are abundant so that the food supporting capacity of the area is reasonably large and is usually adequate unless a very large muskrat population is present. However, at this time coverage appears to be the most critical requirement and may often be the limiting factor.

Errington (1941) reported that muskrats under the stress of hunger would travel considerable distances from water in an effort to obtain food and, when faced with starvation, would eat almost anything including dry grass, bark, or old corn husks. During the winter the muskrat is restricted in its movements and must remain below the ice and snow from early November until late April. At this time it must subsist on what roots, tubers, corms, and rhizomes as may lie within the range of its restricted travels. For this reason winter food is often a limiting factor and this may be expressed in one or more of three ways:

1. The plant population may be so sparse that insufficient food is present.

2. The plants present may be of species with small roots and underground parts containing little nourishment.

3. In the presence of insufficient water and the lack of emergent vegetation to hold the snow, the frost may penetrate into the bottom and cut off the food supply.

Any plant material within reach of the muskrat may be utilized as winter food but those of greatest importance in descending order are as follows:

Typha latifolia
Scirpus sp.
Phragmites maximus
Carex sp.
Sagittaria sp.

There can be little doubt that for quality and quantity of muskrats supported per acre *Typha latifolia* is without a near rival as a native plant type.

Information on winter feeding habits is based on casual observations only, but a few summer feeding experiments were carried out on captive muskrats in an effort to determine food quantity required per day, food preference, and time of feeding. It was found that the specimens were more active during the night and consumed the bulk of the daily food then, but they fed freely during the day as well. The following data are somewhat lower than the findings of Butler (1940) and Collins (1941) but are sufficiently consistent to be of some value:

Scirpus	*Potato*	*Typha*	*Equisetum*	*Carrot*	*Sedge*	*Total*
Specimen no. 1, adult ♂, June 8 to June 22 inclusive						
25.5 ozs.	27.6	58.5	31.5	1.0	11.0	154.1
			Average per day for fifteen days = 10.3 ozs.			
Specimen no. 2, adult ♀, June 13 to June 22 inclusive						
7.0	5.0	54.4	32.0	9.0	3.0	111.5
			Average per day for ten days = 11.1 ozs.			
Specimen no. 3, adult ♂, June 16 to June 22 inclusive						
5.0	0.0	46.0	31.5	4.0	0.0	86.5
			Average per day for seven days = 12.3 ozs.			
Specimen no. 4, adult ♀ plus litter of four young about 24 days old, June 19 to June 23 inclusive						
0.0	0.0	48.0	65.0	0.0	2.5	113.5
			Average per day for five days = 22.7 ozs.			

As illustrated by the above table, the young specimens showed a decided preference for the stalks of *Equisetum* and would take it even though an abundance of other plant material was present. On the basis of these experiments each muskrat would consume about 250 pounds of food material per year. Examination showed that about twelve feet of *Typha* rhizome weighed one pound; thus during one year a muskrat could consume approximately 3,060 feet or .57 miles of rhizome.

In summer food of some sort is reasonably abundant but in winter food appears to be the most critical requirement as the animal does not hibernate or even become much less active. At this time food scarcity will become more evident and the greatest coaction will also take place. A few cases have been observed where a heavy winter population of muskrats largely destroyed the vegetation of the habitat. Winter house building begins around September 1, and it was noted that considerable "mowing" was evident where the muskrat population was dense, but this is of little or no importance as the emergent leaves and stalks used normally die in any case and the future growth of the plants is not injured. The muskrat uses as building material any vegetation present in the vicinity of the selected site, even woody parts, dry grass, or pieces of turf.

One of the main purposes of management then should be to provide and maintain the largest and most adequate specific habitat possible, that is, to provide the greatest muskrat-supporting capacity at all seasons, particularly during the long winter. Observations indicate that the desirable plants grow between a water depth of zero and two feet, so the water levels should be adjusted to give a marginal fringe of greatest area between these depths. It also appears that in order to have optimum water conditions the depth should be greatest in spring and early summer, decreasing gradually toward late summer and rising again somewhat in autumn much as it does in prairie sloughs without permanent streams. If water levels continue to rise during the summer a condition of instability and unhealthiness in plants results.

As previously pointed out, shortly after initial raising of the water level a shift in the distribution of the plants to the new margin is seen, and under suitable water level conditions a new zonation is established in two or three years, and a maximum density is reached in about five years. One would expect this to continue in an unaltered state almost indefinitely but under conditions of consistent high water a deterioration sets in, and in about ten years the area has reverted to open water.

The general opinion is that the land "sours" in time and its productivity is reduced. The one soil element very essential for plant growth and the one most likely to be absent in soils of low fertility is nitrogen. It was thought that the so-called "sour" condition of the soil might refer to a nitrogen deficiency. There are two possibilities here: either the total nitrogen content of the soil is low, or if nitrogen is present in reasonable quantity it may all be taken up during the first four or five years of growth and become bound up in organic form. In the absence of complete decomposition of plant material in the water and mud by bacteria and animal organisms the nitrogen would be found in no simpler form than amino acids. It might be noted that there was an almost complete absence in the water of animal forms such as Cladocera and Copepoda, and bottom dwelling forms such as chironomid larvae were quite rare.

During the summer of 1947 seventeen samples of mud and water from representative parts of the area were analysed for total nitrogen content, but it was impossible at that time to check the presence of nitrogen in the form of ammonia, nitrites, or nitrates. The results showed the pure clay to be devoid of nitrogen in any form, but some samples of black mud from amongst dense vegetation showed a total nitrogen content of as high as 0.44 per cent. It is essential to know what portion of this is available to plants in the form of simple salts, etc., as it is suspected that complete breaking down of the organic material does not take place in the absence of sufficient air and in the submerged condition. If the soil becomes depleted of nitrogen in this way it may be necessary to drain the marshes periodically to restore their fertility.

Three sub-species of muskrats have been recorded from Manitoba, (Anderson, 1946), but only one, *Ondatra zibethica alba*, the Hudson Bay muskrat, is abundant and widely distributed. It is the sub-species concerned in this study. During the summer twenty-eight litters of young involving 133 known individuals were observed. In many cases only a single litter was seen but in four cases second litters were known. First litters arrived from May 29 to June 17, while second litters arrived from June 26 to July 28, with a time interval between litters of from twenty-five to thirty-four days. Our findings agree closely with those of Errington (1937) which set the gestation period of the muskrat at about twenty-five days with an interval between successive litters of thirty days.

Litter sizes ranged from one known individual to a maximum of twelve with the average size of first litters being 5.0 and of second litters 5.6 young. This corresponds favourably with 6.5 for Iowa (Errington, 1939), 4.4 for Maryland (Smith, 1938), and 3.8 for Louisiana (Arthur, 1931). No evidence that a third litter was produced in one season was seen in Manitoba or that the young of the first litter reproduced the same year although the evidence so far is not conclusive. While litter size in Manitoba compares favourably with that in other places the general reproductive rate is somewhat lower because of the shorter breeding season and the fewer numbers of litters per year. About the end of July the majority of adult muskrats and weaned young appear to vacate the breeding houses and disperse through the marsh. Until winter house building begins about September 1, they apparently have no permanent abode but sleep on feeders, in improvised houses, or in otherwise abandoned houses which they encounter in their travels.

The present study, based on observations of areas which had been flooded for various lengths of time up to ten years, indicates that there is a general rise in the muskrat-supporting capacity of a marsh up to the fourth or fifth year following flooding. This is then followed by a gradual decline until at the end of about ten years the supporting capacity is again low. Other factors being equal it is natural to expect the muskrat population to follow closely the supporting capacity of the habitat but a consideration of census figures based on winter house counts over a period as long as ten years shows that this is not the case. The following graphs (Figs. 4 and 5) give a comparison of the numbers of winter muskrat houses on smaller zones within the larger area from year to year, and is considered to be a more reliable index of population than

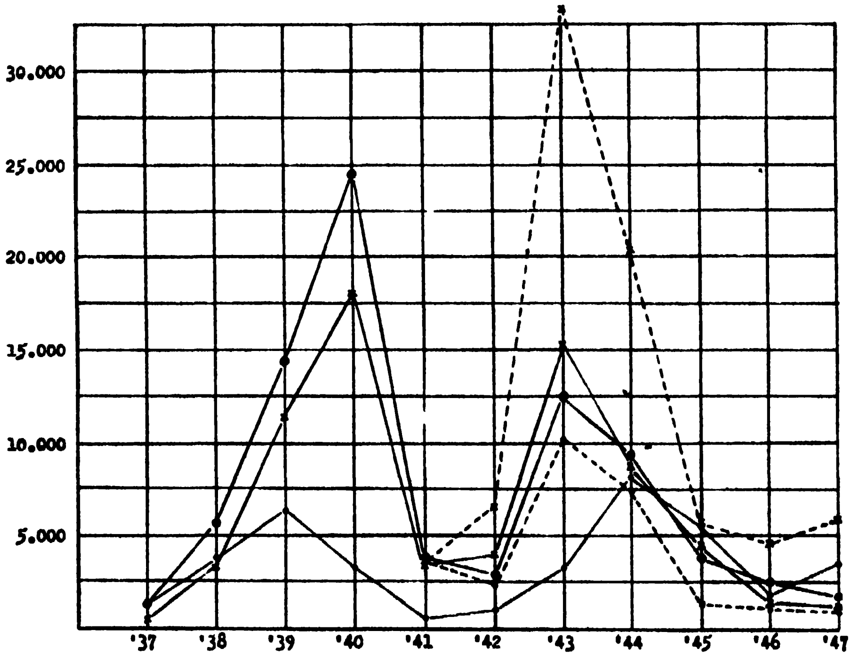


FIGURE 4.—Graph showing muskrat house numbers in three zones of Area *A* (solid line) compared to those of two zones of Area *B* (broken line).

spring trapping returns although, admittedly, it is open to some question. These data indicate that the maximum population is governed largely by the supporting capacity of each zone, but that a definite increase and decrease in the population occurs in five years and shows a tendency to be repeated in a cyclic manner. For purposes of comparison, zones as far removed as forty miles and in different stages of flooding and plant development were taken. While the rises and falls in population numbers are not exactly synchronous there appears to be sufficient similarity to indicate a general trend. Some increases are known to have continued in the face of heavy trapping, while decreases also have been known to occur in the absence of any trapping.

Such sharp declines in population might be explained on the basis of lowered reproduction or increased mortality due to starvation or predation, but information from field observers indicated that the decline may occur under very favourable conditions as regards food when the animals are in good nutritional condition. Such information states that periodically a pronounced die-off occurs, and that dead animals are found in great abundance. These specimens are reported to be fat and to have been feeding normally but to have died suddenly without evidence of sickness or distress. What small amount of material has come to hand for post-mortem examination strongly indicates a virus enteritis, first reported by Errington (1946) and referred to in many quarters as "Errington's disease."

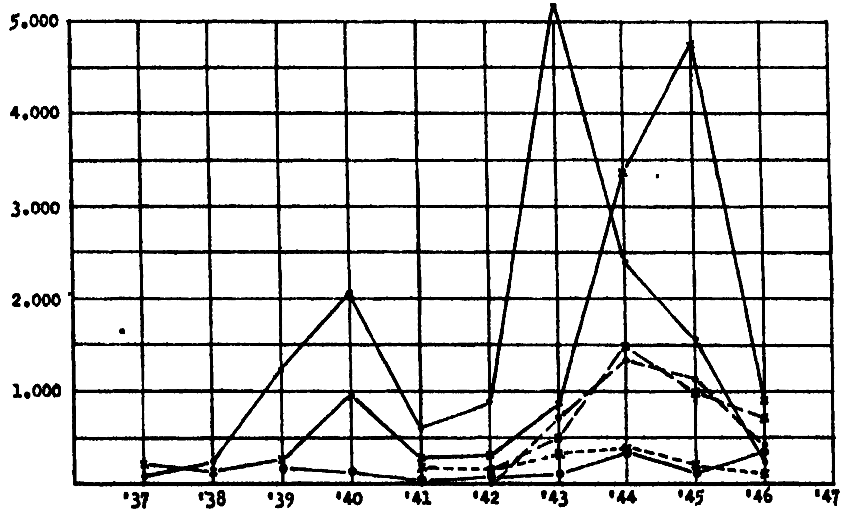


FIGURE 5.—Graph showing muskrat house numbers in six zones some of which are from widely separated areas.

In addition to the foregoing brief studies much work will be necessary to prove that definite cyclic fluctuations in muskrat population do occur and, if so, to determine the cause or causes. At the moment it would appear that a virus enteritis is responsible for the increased mortality which results in the sharp decline. Why the die-off should recur with regularity regardless of food conditions or population density is a matter for speculation. It seems probable that the virus may be present all the time and that the variable is host resistance resulting from a vitamin or trace element deficiency in the diet.

CONCLUSIONS

On the basis of brief studies which have been carried out to date the following conclusions may be tentatively arrived at, but it is pointed out that much additional study is necessary before they can be accepted as fact:

1. The specific habitat of the marsh muskrat is the marginal fringe of emergent vegetation from the edge of the marsh or slough to open water.
2. The supporting capacity of this area increases for from four to five years following flooding, and then decreases during the ensuing five years until it again becomes very low.
3. The indications are that the decline in vegetation results from a nitrogen deficiency in the soil.
4. A maximum of two litters of young muskrats per year was found.
5. No evidence was found that the young of the first litter reproduce the same year.
6. The average size of muskrat litters in Manitoba was about 5 to 5.6 in 1947.
7. The adult muskrat consumes, on the average, twelve ounces of living plant material per day.
8. Muskrat populations in Manitoba appear to show cyclic fluctuations with a frequency of five years.
9. A periodic epizootic of virus enteritis is thought to occur.
10. The epizootic may result from a vitamin or trace element deficiency in the diet.

ACKNOWLEDGMENTS

The writer wishes to acknowledge with thanks the kind assistance of the staff of the Game and Fisheries Branch, Department of Mines and Natural Resources, Professors R. A. Wardle and W. Leach, and Mr. D. R. Moir, University of Manitoba, and Messrs. S. L. Baldwin and M. Levin.

REFERENCES

ANDERSON, R. M. (1946). Recent Canadian mammals. Nat. Mus. of Canada, Bull. no. 102, Biol. Ser., no. 31.

ARTHUR, S. C. (1931). The fur animals of Louisiana. Louisiana Dept. of Conservation, Bull. 18

BUTLER, L. (1940). A quantitative study of muskrat food. Can. Field Nat., **54**: 37-40.

COLLINS, H. (1941). Unpublished data.

ELLIS, J. H. (1938). The soils of Manitoba. - Manitoba Economic Survey Board. Pp. 112.

ERRINGTON, P. L. and ERRINGTON, C. S. (1937). Experimental tagging of young muskrats for study. J. Wildlife Management, **1**: 49-61.

ERRINGTON, P. L. (1939). Observations on young muskrats in Iowa. J. Mamm., **20**: 465-478.

—— (1941). Versatility in feeding and population maintenance of the muskrat. J. Wildlife Management, **5**: 68-89.

—— (1946). Special report on muskrat disease. Quarterly Report, July-September, 1946, Iowa Cooperative Wildlife Research Unit, pp. 34-51.

HALLIDAY, W. E. D. (1937). A forest classification of Canada. Can. Dept. Mines and Resources, Forest Service Bull. 89.

SMITH, F. R. (1938). Muskrat investigations in Dorchester County, Maryland, 1930-34. U.S. Dept. Agriculture, Circular 474. Pp. 24.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Fecundity and Mortality in Pacific Salmon

By FERRIS NEAVE

Presented by R. E. FOERSTER, F.R.S.C.

THE five species of Pacific Salmon which inhabit British Columbia exhibit marked differences (which have been duly noted by previous writers) in the average number of eggs which they produce.

While admitting the obvious fact that large fluctuations in the abundance of these fish take place from year to year due to natural causes, and the possibility that fishing operations may have induced a longer-term reduction in numbers in some instances, it must still be assumed that the average number of eggs deposited by a female is closely adjusted to the hazards of the life history. Although the level of abundance of the whole population may change from time to time, any continued under-production or overproduction of eggs by the average individual would quickly change the whole status of the species.

Much effort has been, and is being, expended on the slow accumulation of information concerning the details of the life history of these species, particularly the causes and extent of mortality at various stages of development. Most of these investigations have as their ultimate aim the provision of a scientific basis for methods of maintaining or increasing the stocks of salmon. It is of interest, therefore, to compare the life histories of different species in the light of our present fragmentary knowledge, and to see to what extent the known or presumed facts can be fitted to the fecundity, which, as already mentioned, should represent a measure of the total hazards attending the life cycle.

For example, from Table I it would appear that the egg of a pink salmon has twice as good a chance of completing the cycle as the egg of a sockeye. Coho and chum salmon show a very similar average egg-count yet differ markedly in life history. Can such facts be explained?

Egg Production

The spring salmon (*Oncorhynchus tshawytscha*) has been omitted from consideration at the present time, for although some egg-counts are available for this species in British Columbia there is insufficient information regarding mortality at any stage to provide a basis for examination. Egg-counts for pink salmon (*O. gorbuscha*), chum (*O. keta*), coho (*O. kisutch*), and sockeye (*O. nerka*) are presented in the following table. These figures have been taken from Foerster and Pritchard (1936), with the addition of data acquired by the Pacific Biological Station in more recent years.

In spite of the not inconsiderable variation which is apparent in egg-counts from different localities, the four species fall into three well-marked groups with averages of roughly 1,700, 2,700, and 3,700 eggs respectively.

TABLE I

Species	*Locality*	*Average no. of eggs*
Pink	McLinton Creek (6 yrs.)	1,719
	Morrison Creek (2 yrs.)	1,820
	Port John Creek (1 yr.)	1,520
	Namu (1 yr.)	1,841
	Fraser (1 yr.)	1,755
	Average of all localities	1,731
Chum	Nile Creek (3 yrs.)	2,726
	Port John Creek (1 yr.)	2,335
	Namu (1 yr.)	2,760
	Fraser (1 yr.)	2,943
	Average of all localities	2,691
Coho	Cowichan (9 yrs.)	2,329
	Port John (1 yr.)	2,313
	Namu (1 yr.)	3,002
	Fraser (1 yr.)	3,152
	Average of all localities	2,699
Sockeye	Babine (1 yr.)	3,287
	Nass (1 yr.)	3,461
	Namu (1 yr.)	3,264
	Fraser (1 yr.)	4,180
	Cultus (6 yrs.)	4,086
	Average of all localities	3,656

MORTALITY

In considering the significance of these differences, the probable main causes of mortality during the life cycle can be listed as physical and chemical conditions in the environment, infections (fungus, bacteria, etc.), predation, insufficient food supplies. Non-fertilization of the eggs is not covered by these categories. Its importance has been seriously questioned, and in any case it has not been shown that there are significant differences between species in this respect. The previously-mentioned factors do not exert equal pressure at all stages in the life-cycle.

Physical and chemical conditions, as causes of actual mortality, are presumed to exert their effects mainly in fresh water, where there is abundant evidence of destruction associated with changes in water level and stream velocity. Variations in temperature and oxygen content are also relatively great in fresh water, and may directly or indirectly influence survival. Freshwater conditions, of course, affect not only the eggs and young fish, but also the adults for a period preceding spawning.

The importance of fungal and bacterial infections has not been evaluated. It may not be great as a direct cause of differential mortality between species. However, a very heavy incidence of Saprolegnia has been observed among chum salmon eggs in certain streams.

Loss by predation takes place throughout the life-cycle but is especially prevalent among the fry and smaller fish. The close inverse relationship which exists between mortality and size has been shown by Kelez (1937), Foerster (1938), Needham (1938), and others. Factors tending to reduce the growth rate can therefore be expected to have an unfavourable effect on survival, by prolonging the most vulnerable stage. Food supplies are presumably important in this connection.

Direct counts of populations of young salmon (thus permitting estimates of preceding mortality) have been made so far only at periods when the fish are migrating and can thus be readily trapped. Such counts are available in a number of instances for pink, chum, and coho salmon at a period immediately after the free-swimming state has been reached, and for sockeye during the seaward migration (after at least one year in fresh water). These data are summarized in the following table, which shows the percentage survival up to the time of counting, based on the number of eggs available for deposition by the adults which entered the area. Most of these data have been published by Pritchard (1947).

TABLE II

Species	*Locality*	*Stage*	*No. of experiments*	*Average per cent survival*
Pink	McClinton Creek	fry	6	14.4
"	Morrison Creek	fry	2	5.7
Chum	Nile Creek	fry	3	1.26
Coho	Cowichan Lake	fry	12	22.8
Sockeye	Cultus Lake	yearling	3	1.8
"	Lakelse Lake	yearling	1	1.1

COMPARISON BETWEEN SPECIES

Pink salmon

This species, which has the lowest average egg-count, presumably has also the lowest mortality over the whole life-cycle. The evidence presented in Table II indicates that the average percentage survival is neither very high nor extremely low (possibly in the neighbourhood of 10 per cent) up to the time of going to sea. Since the latter event takes place immediately after emergence from the gravel, the young fish are released at the earliest possible stage from the restricted food supplies and numerous predators of the freshwater environment. While predation undoubtedly continues in the ocean, it may be presumed that rapid growth quickly reduces its intensity.

Another factor which may benefit the pink salmon is the shortness of the life-cycle, which reduces to two years the period in which all mortality must occur.

The pink salmon is not characteristic of streams which are liable to excessive scouring or drying. It is suggested that, because of the limited production of eggs, pink salmon can only maintain their numbers by spawning in localities where destruction by physical conditions is not extremely high.

Chum salmon

According to Table I, a female chum produces an average of about 1,000 more eggs than a female pink. Like the latter species, the free-swimming fry leave the streams immediately. It is evident that if the relative abundance of the two species is to remain constant, the mortality of chums during the life cycle must exceed that of pinks by about 40 per cent. Since chums do not mature until three or more usually four years of age, all mortality occurring during the last one or two years of life would tend to reduce the discrepancy. Although experimental evidence on this point is admittedly lacking, it seems unlikely that the mortality occurring after the attainment of a size representing two seasons' growth in the sea would account for the difference in the initial number of eggs. On the other hand, there is evidence that chum salmon eggs and alevins are apt to suffer greater losses than pinks. Although the two species often spawn on the same grounds, chums frequently occupy streams or portions of streams in which physical conditions are apt to become very severe and from which pinks are virtually absent. In small coastal streams, the parent fish commonly reach fresh water in an advanced condition of maturity, and are less able than pinks to overcome rapids and falls and reach desirable spawning grounds upstream. Many spawn in areas near the river mouths, where shifting and drying of the bottom are frequent occurrences.

In three experiments at Nile Creek, Vancouver Island, the percentage survival of chums at the time of migration to sea was only 3.0, 0.44, and 0.38. These percentages are probably below the general average, since destructive floods are frequent in this stream. It is almost certain that the two lower recorded figures would fall far short of maintaining the stock. The inference is drawn, however, that the eggs and alevins of chums are frequently subjected to losses which would more than offset the initial superiority which this species possesses over the pink salmon in the number of eggs produced. A final balancing of the account would have to recognize that chum salmon fry are larger than the corresponding stages of pink salmon, and may therefore be presumed to have a somewhat better chance for survival.

Coho salmon

The female coho produces approximately the same number of eggs as the female chum. In contrast with the latter species, the survival up to the free-swimming stage appears to be relatively very high. Although all the twelve experiments recorded in Table II were performed in only two closely adjacent streams, these are not considered to present unusually favourable conditions in comparison with other coho spawning grounds. The high survival rate of the early stages can be attributed at least in part to the marked ability of the adult fish to overcome the difficulties of upstream travel and thereby reach spawning grounds where physical conditions are relatively stable. In comparison with the chum the coho possesses the further presumed advantages of a (frequently) shorter life-cycle and a higher growth rate during the salt water phase.

Since survival to the free-swimming stage appears to be much greater, and since ocean survival can be assumed to be greater also than in chums (in view of the shorter period spent in salt water and the much larger size at which cohos enter the sea), equalization in numbers must take place during the year which the young fish spend in fresh water. During this period they are exposed to dangers by predation, drouth, and possibly by limited food supplies from which the chum escapes. These dangers may be increased by the frequent necessity of undertaking a relatively long downstream journey.

The marked effect on coho populations of stream conditions at a period subsequent to the exodus of chums can be illustrated by reference to the Cowichan River. Here, the abundance of returning adults appears to vary with the minimum late summer stream flow two years previous, that is, when these adults were fingerlings in fresh water (Neave, 1948).

It may be speculated that a young chum about to enter the sea is the equivalent in survival value of at least four or five cohos at a similar stage of development (assuming a previous survival of 20 per cent to 25 per cent in cohos, and 6 per cent or less in chums.

Sockeye salmon

Since the adults of this species are very active migrants and usually spawn in locations where physical conditions are relatively favourable, it is likely that survival up to the free-swimming stage is relatively high. The sockeye, however, spends a year (sometimes longer) in fresh water. Moreover the fry are very small in size and therefore presumably highly vulnerable. The length of the freshwater migration is often great and the life-cycle is relatively long (usually four to five years). The sockeye therefore faces the same hazards as the coho in an exaggerated degree.

The very high losses occurring during a year's residence in a lake have been shown by Foerster (1938), who found that only about 4 per cent to 11 per cent of planted fry migrated downstream. The percentage survival from potential natural deposition to seaward migrants has been estimated at from 1 per cent to 3 per cent by Foerster at Cultus Lake (1938) and Pritchard at Lakelse Lake (1947), these counts being made at points where the fish were still a considerable distance from the sea.

In view of these considerations it appears entirely reasonable that the sockeye should require a numerically higher egg production than the three species discussed previously.

The egg-producing capacities of these four species of salmon appear to confirm the available information on the hazards of their respective life histories, and to emphasize the differences between these species in their reaction to environmental pressure. It is indicated that only the species having a high or fairly high egg production and relatively favourable conditions for incubation and alevinage can withstand the mortality imposed by a prolonged freshwater existence. Conversely, species with a low egg-production or more hazardous spawning-ground conditions must escape to sea at an early stage in order to maintain themselves.

EFFECTS OF ENVIRONMENTAL CHANGES

The maintenance of a certain general level of abundance through the balancing of fecundity and mortality of course does not preclude very considerable annual fluctuations. In addition to these, however, longer term changes in the environment sometimes occur. These may be expected to raise or lower the average level of abundance. Moreover, in view of the different habits exhibited by the various species, such changes might affect some more than others and thus cause changes in relative as well as in absolute abundance.

In many of the coastal streams and small rivers draining into the Strait of Georgia, destructive floods have occurred with notable frequency during the past ten or fifteen years. For example, between 1916 and 1933, the recorded discharge of the Campbell River only once exceeded 18,000 cubic feet per second. This figure was exceeded in five out of the following ten years. Low water levels also appear to have been more prevalent in the nineteen thirties and nineteen forties than at an earlier period. The late summer discharge of the Cowichan River during the period 1940-6 averaged much less than during the period 1914-19. While actual comparative measurements are available in only a few instances, it is believed that there has been a widespread deterioration in stream conditions in this region. Deforestation is strongly suspected of having contributed to these changes, which could therefore be expected to occur in other coastal areas in the future. Whether or not this is admitted as the sole cause, a period of increased fluctuation in the run-off might be expected to produce certain effects on the pinks, chums, and cohos which constitute the main salmon populations of these streams.

1. Lower water conditions in early fall would more especially increase the difficulties and hazards of pink salmon in reaching spawning grounds, since this species tends to enter streams before they have become swollen by autumn rains.

2. Greater frequency and severity of floods and drouths would increase the destruction of eggs and alevins in pink and chum spawning grounds. It is possible, however, that in certain localities, chums might be able to take over areas formerly occupied mainly by pinks and still maintain themselves by reason of their greater egg production. This presupposes some degree of competition for spawning grounds between these species.

3. Coho fry production might be less quickly influenced by changes in the run-off, but fingerlings (which tend to spread throughout a stream system) would be adversely affected by reduced stream flow in summer and early autumn.

Available information on the salmon runs to certain streams of southeast Vancouver Island tends to support these interpretations of cause and effect. In recent years, the runs of pink salmon in Amor de Cosmos Creek (Bear River), Oyster River, and certain streams in the Qualicum area have become greatly reduced—in some instances almost to the vanishing point. Incidentally, these are areas in which much deforestation has taken place. Relatively good runs of chum salmon still occur in these streams, although marked fluctuations, apparently correlated with annual variations in stream flow, are evident. In some years, conditions are severe enough to be disastrous for this species, too.

In the case of the coho, reference has already been made to the apparent effect of low water on the return of Cowichan River fish. Fishermen have made numerous complaints of a reduced abundance of this species in the general area under discussion.

PROBLEMS IN SALMON CULTURE

A comparison of the kind attempted herein suggests that attempts to increase production might well be varied to suit the species. Although it can reasonably be argued that any increased resistance to environmental pressure at any stage would benefit any species, it appears that, *in relation to the others*, each species has certain weaknesses. The elimination or reduction of these could confer great advantages without going beyond conditions already in existence for these other species. For example, there seems to be no *a priori* reason why chum eggs could not be sufficiently protected to produce a percentage fry output comparable with pinks or even cohos. At least two methods would seem to be practicable on a large scale and at relatively low cost:

(*a*) The establishment of beds with a controlled water supply in which natural spawning or planting of green eggs could be conducted.

(*b*) The establishment of eyeing stations in which artificially fertilized eggs could be held during the greater part of the incubation period, which is also the period when losses are usually greatest under natural conditions.

The first method offers advantages in that protection from detrimental physical conditions is afforded to alevins as well as to eggs, and the trouble and expense of caring for the eggs during several months are avoided. The second method permits closer observation of the developing eggs, with better opportunities for control of fungus, etc. A combination of the two procedures might give still better results. These methods are being tested at present by the Fisheries Research Board.

Similar measures might well be applied to pink salmon in which species the losses, while somewhat less excessive, also tend to occur in the early stages.

As regards the coho and the sockeye the ideal method of increasing survival would be the elimination of the long period of residence in fresh water. There seems to be no conclusive evidence as to the feasibility of acclimatizing the fry to salt water. If this could be accomplished, it might lead to a far more effective form of fish culture than a routine hatchery operation. Failing this, the provision of storage dams for the purpose of increasing summer flow should benefit the coho in certain areas. The general desirability of stream flow regulation for all species is, of course, evident, but the application of structural controls to any considerable proportion of the numerous salmon streams is likely to be severely limited by costs.

On the biological side there are certain possibilities which are purely imaginative at the present time but may eventually be deemed worthy of exploration, as for example the selective breeding of salmon for higher egg production (the pink salmon with its short life-cycle would evidently be the most suitable species for experiment).

Foerster (1935) has shown that cross-breeding of species is feasible and he has speculated on the possible advantages with reference to such characters as size and quality. It may be added that a hybrid might well have an increased survival value if, for example, it combined the early-migrating habit of the chum with the large fry output of the sockeye.

REFERENCES

FOERSTER, R. E. (1935). Inter-specific cross-breeding of Pacific salmon. Trans. Roy. Soc. Canada, **29**, sec. V: 21-33.

———(1938). Mortality trend among young sockeye salmon (*Oncorhynchus nerka*) during various stages of lake residence. J. Fish. Res. Board of Canada, **4**: 184-191.

FOERSTER, R. E. and PRITCHARD, A. L. (1936). The egg content of Pacific salmon. Prog. Repts. Pac. Coast Stations, **28**: 3-5.

KELEZ, G. B. (1937). Relation of size at release to proportionate return of hatchery-reared coho (silver) salmon. Progressive Fish. Cult., no. **31**: 33-6.

NEAVE, FERRIS (1948). Game fish populations of the Cowichan River (MS).

NEEDHAM, P. (1938). Trout streams. Ithaca, N.Y., Comstock Publishing Co.

PRITCHARD, A. L. (1947). Efficiency of natural propagation of Pacific salmon. Can. Fish Culturist, **1**: 22-6.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Giant Axons of *Eudistylia vancouveri* (Kinberg)

By J. A. COLIN NICOL

Presented by W. A. CLEMENS, F.R.S.C.

THE giant axons of polychaete worms differ so much from typical neurones that further information concerning their structure and arrangement is highly desirable. Apart from their large size they show many interesting peculiarities (for example, segmental septa and syncytial conditions) that require further exploration and study. Moreover, the large size of these axons is itself of great inherent value in that it permits technical procedures to be carried out and critical observations to be made, that would be difficult or impossible on axons of more usual size. Our knowledge of the giant axons of polychaetes is still too fragmentary to give us a generalized picture of the pattern or patterns of giant nerve fibres in this order. Of the various families of Polychaeta possessing giant axons those of the Sabellidae are probably the most striking and best known, and it is possible within this group to trace some general pattern of arrangement. In order to provide additional information about the giant nerve fibres of polychaetes, particularly the Sabellidae, I have made a study of the large Pacific species, *Eudistylia vancouveri* (Kinberg), the results of which are here presented.

OCCURRENCE AND HABITS

E. vancouveri occurs from Alaska to central California (Bush, 1910; O'Donoghue, 1923; Berkeley and Berkeley, 1942; Hartman, 1942). According to Bush and O'Donoghue the species is usually solitary but in Vancouver harbour it occurs in enormous numbers where clumps of sixty to one hundred tubes form "groves" in the vicinity of low spring-tide mark. I have obtained my material from the shore at Stanley Park, Vancouver. The animals live in tough parchment-like tubes which stand upright, the lower third, being buried in the mud, or fixed in rock crevices. At low tide nearly all the animals are retracted into their tubes although a few still have their branchial crowns protruding. With crown withdrawn the mouth of the tube usually becomes closed by the apposition of two sides forming lip-like flaps. When undisturbed in sea water the animal protrudes its large feeding crown from the mouth of the tube, but retreats instantly on stimulation, which may be tactile, vibrational or photic (viz., a passing shadow).

GENERAL ANATOMY

This species is relatively large, up to 50 cm. long, but the absolute length depends on the state of contraction or expansion and constantly changes. It is about 17 mm. wide in the thoracic region. There are about three hundred segments, eight in the thorax. Bush (1910), Hartman (1938, 1942), and O'Donoghue (1923) have described the specific characteristics, and the latter author has given a brief account of the internal anatomy. He has noted that the body wall is very thick and muscular. Six longitudinal muscle blocks are present: two small medio-ventral, two ventro-lateral, and above these a pair of large dorso-lateral muscle blocks. These masses are composed of longitudinal muscle fibres and they extend through the length of the animal. They are separated from one another by grooves containing coelom. A layer of circular muscle lies beneath the epidermis, external to the longitudinal musculature. The alimentary canal is suspended within the body cavity by dorsal and ventral mesenteries. The ventral mesentery extends from the ventral face of the gut to the bottom of the groove between the two ventral muscle blocks.

O'Donoghue (1923) has described also the character of the nerve cord. He found a double nerve cord lying in the ventral muscle blocks at the bottom of the median ventral groove. He stated that each cord consists of a central mass of fibres bounded laterally and ventrally by nerve cells ("ganglion cells"), and that a band of fibres extends between the two separated cords. "In the connective tissue internal to the median corner of each cord lies a well marked blood vessel."

I have been able to confirm O'Donoghue's description of the anatomy of the body wall and alimentary canal. I find, however, that in common with other sabellids, the ventral blood vessel is unpaired and lies within the ventral mesentery (Ewer, 1941; Nicol, 1948). I have studied the nerve cord in detail and I find conspicuous giant axons in place of the two ventral blood vessels described by O'Donoghue.

HISTOLOGICAL METHODS

I have employed the following standard solutions as fixatives: Bouin's fluid, Helly's fluid (or Zenker-formol), Flemming's strong solution, picro-formol, and picro-osmic. Bouin's fluid was made up in sea water, as recommended by Young (1935). Flemming's solution contained 32 g.

NaCl per litre. The picro-formol consisted of a saturated solution of picric acid in sea water, 90 parts, and commercial formalin, 10 parts. Picro-osmic consisted of a saturated solution of picric acid in sea water, 80 parts, and a 1 per cent solution of osmic acid, 20 parts. Subsequently the specimens were embedded in paraffin for cutting histological sections. For more critical work I have used Peterfi's double embedding method.

The following staining methods proved of value for revealing the giant axons and nerve cells of *Eudistylia:* Heidenhain-azan, Masson's triple and Mallory's triple stains, brazilin-aniline blue, Harris's heamatoxylin, and eosin. Holmes's (1947) silver method was particularly valuable for neurone impregnation after Bouin and picro-formol fixation. This method involves incubation in a buffered pyridine-silver bath; silver concentrations varied from 1/9,000 to 1/20,000. Bodian's activated protargol was also used with success.

Histological procedures used for the demonstration of myelin comprised: fixation in picro-osmic (Holmes, 1942), and fixation in Baker's formol-calcium and "staining" of frozen-cut sections with Sudan black (method in Pantin, 1946).

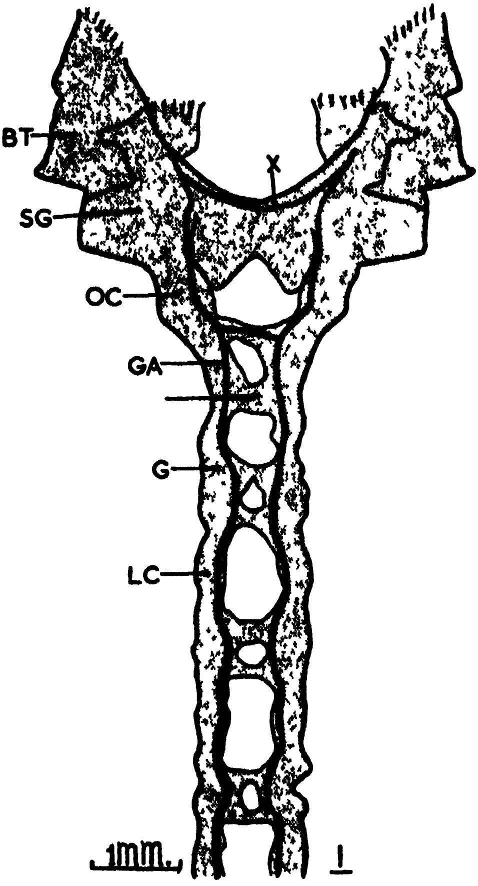
OBSERVATIONS

The giant axons of *Eudistylia vancouveri* lie within the central nervous system. In order to appreciate their position, therefore, it has been necessary to determine the general morphology of the brain and nerve cord. Graphic reconstructions of these organs were made.

General morphology of the central nervous system

The supra-oesophageal ganglia lie above the gut in the prostomium, and extend back into the first setigerous segment. They form a short broad mass, three times the breadth of the thoracic cord. The main mass of the brain is composed of two large lobes lying dorso-lateral to the oesophagus and connected above the gut by a thick commissure (Fig. 1). These lobes extend posteriorly into setiger I and dorsally on either side of the excretory duct of the cephalic nephridia. Anteriorly each lobe is connected with a large branchial nerve tract lying above it. The latter forms a broad mass beneath the branchial skeleton on either side of the head. The two tracts really constitute roots of the branchial nerves since they divide into radial branches entering the filaments of the crown. The arrangement of nerve cells is typical of polychaetes, with cell bodies lying peripherally about a central mass of nerve tracts and neuropil.

The nerve cord consists of two longitudinal divisions running through the entire length of the trunk (Fig. 2). Anteriorly each division extends for a distance of almost 1 mm. immediately beneath the supra-oesophageal ganglia before finally joining these structures. The oesophageal connectives are short downward projections of the brain Each division of the cord remains connected with the supra-oesophageal ganglia for a distance of about 300μ, then passes forwards independently into the ventral region of the prostomium. This forward extension of the nerve cord into the head is correlated with the strongly developed prostomial



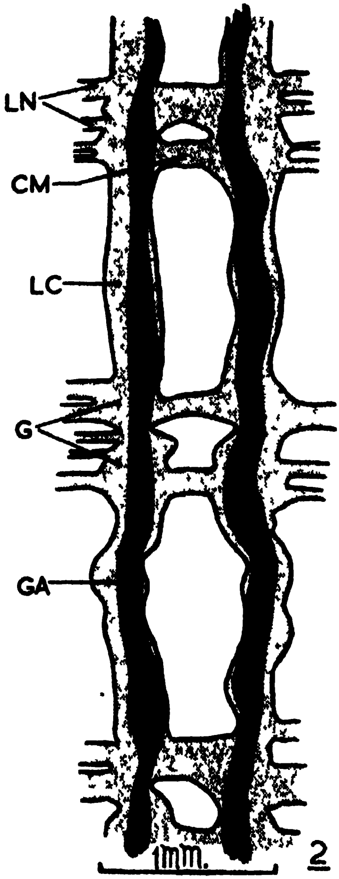


FIGURE 1.—Graphic reconstruction of the anterior central nervous system of *Eudistylia vancouveri*. Outline only, ventral view.

FIGURE 2.—Graphic reconstruction of the thoracic nerve cord of *Eudistylia vancouveri*.

Legend. *BT*, branchial tract; *CM*, commissure between ganglia of cord, *G*, ganglia of nerve cord, *GA*, giant axon; *LC*, longitudinal connective, *LN*, peripheral nerve, *OC*, oesophageal connective; *SG*, supra-oesophageal ganglia, *X*, branches of giant axons in dorsal commissure of supra-oesophageal ganglia.

musculature which forms broad masses lying ventral and lateral to the branchial skeleton. Nerves proceed laterally from the nerve cord to the muscles of this region.

The arrangement of the nerve cord in the thorax and abdomen is very similar to that obtaining in *Sabella* and *Spirographis* (Meyer, 1887, 1888; Thomas, 1940). There are two pairs of ganglia in each segment, one pair in the anterior region, the other pair in the posterior region (Fig. 2; Plate I, Figs. 1-4). The interval between posterior and anterior pairs of two adjacent segments is very short, while long connectives join the anterior and posterior ganglionic pairs of the same segment. Members of each pair of ganglia are connected together by a commissure. The nerves proceeding to the body wall are segmentaly arranged also, taking their origin from the ganglia. Two or three nerves arise from each ganglion. The largest nerves extend ventro-laterally into the body wall beneath the longitudinal muscles. A smaller nerve also arises above these from the lateral face of each ganglion, and extends into the medio-ventral muscle block (Plate II, Fig. 3). In addition, numerous small nerves arise from the ventral surface of the cord throughout its length, and enter the thick glandular epithelium.

The nerve cord is surrounded by a relatively thick sheath of connective tissue (Plate I, Fig. 4). The sheath consists of collagenous material in the meshes of which there are cells, blood vessels, and some longitudinal muscle fibres on the lateral faces.

Within the nerve cord occur nerve cells, neuropil and nerve tracts, supporting cells, and the giant axons with their branches. The nerve cells, confined to ganglia, lie ventrally and laterally beneath the neuropil (Plate I, Figs. 3-5). The latter contains not only an intricate intermingling of nerve cell processes, but nerve tracts as well, extending longitudinally through the nerve cord, transversely through the commissures, and centrifugally and centripetally through the peripheral nerves. The supporting cells form a definite type of neuroglia-like tissue. The cells are small and stellate or fusiform in shape. The scanty cytoplasm invests a small oval nucleus and continues out into branching processes which extend considerable distances in the nerve cord. The cytoplasm stains feebly with all stains employed. Fine intracellular fibrils become visible after treatment with iron haematoxylin; they continue into the cellular processes and probably beyond the boundaries of the cell. The individual cells with their processes form thin but discrete capsules investing nerve cells and constituting a network in the meshes of which lie individual nerve fibres and groups of nerve fibres. The supporting cells of the cord are not unlike vertebrate neuroglial cells in some respects, in their refractoriness to stains, in their branching stellate form, and in their relation to nerve cells and fibres.

Similar supporting cells occur in the nervous system of *Myxicola* (Nicol, 1948), and Havet (1916) has described cells, which he called fibrous and protoplasmic glial cells in the nerve cord of *Lumbricus*. According to Havet these cells are associated both with blood vessels and neurones. In *Myxicola* and *Eudistylia* however blood vessels do not

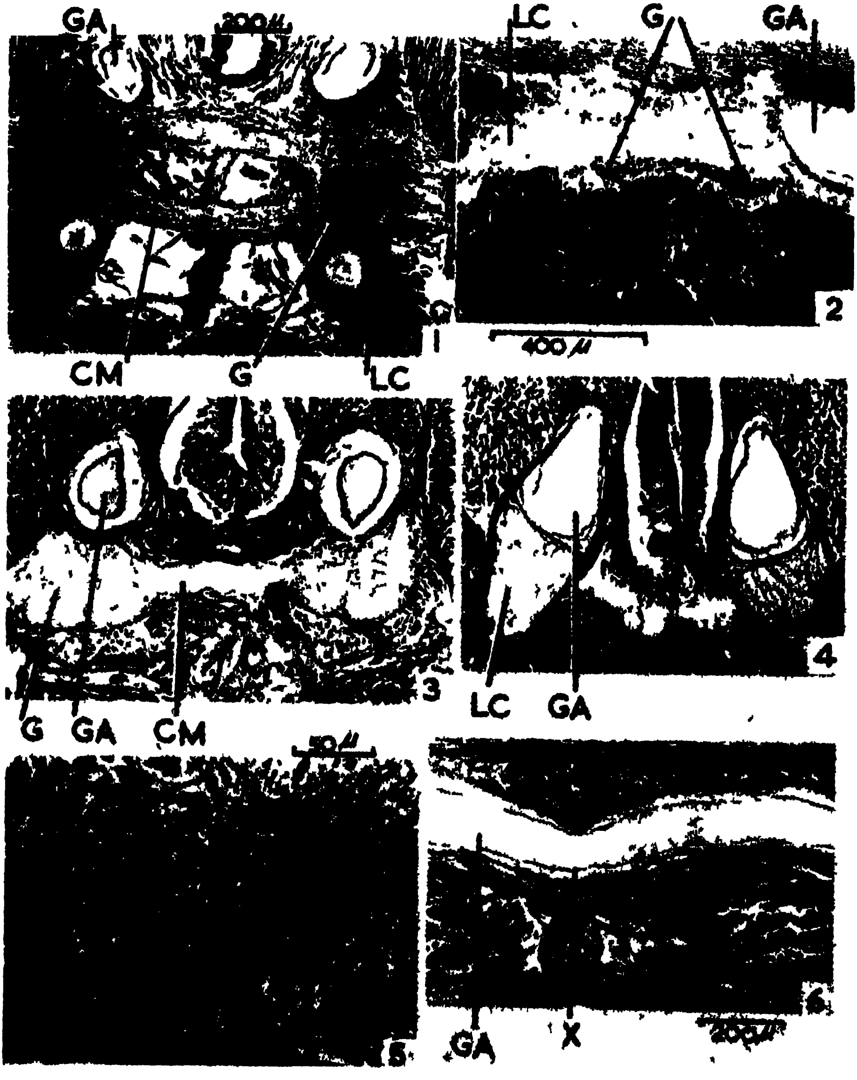


PLATE I

enter the nerve cord; consequently the supporting cells in these animals do not play any obvious functional role in the trophic relations of nerve cells and capillaries, as suggested for vertebrates.

Arrangement of the giant axons

There are two giant axons in the nerve cord of *Eudistylia*. They are conspicuously large and extend from the anterior prolongations of the nerve cord in the prostomium to the posterior end of the animal. One axon lies in the dorsal region of each division of the cord and is separated from the nerve cord proper by a horizontal septum. In appearance the giant axon lies in a separate compartment of its own above the nerve cord, and is surrounded by a connective tissue sheath continuous with that of the cord. The sheath is interrupted ventrally within the ganglia, thereby permitting branches of the giant axon to descend into the nerve cord. Towards the posterior end of the animal the separation between nerve cord and giant axon is less distinct. (Plate I, Figs. 3, 4; Plate II, Figs. 3, 4). Anteriorly, branches of the giant axon pass dorsally into the supra-oesophageal ganglia. Here giant axon branches from either side of the animal meet in the commissure connecting the lateral lobes of the brain above the gut.

In a mature specimen the giant axon branches have a diameter of about 4μ in the supra-oesophageal ganglia, 4μ in the dorsal, and 40μ in the ventral regions of the oesophageal connectives. In the suboesophageal ganglia the axons are 80μ in diameter, and increase to 90μ in the anterior thorax. They are largest (about 250μ) in the posterior thorax and anterior abdomen, they decrease in diameter posteriorly, and in the ter-

PLATE I

Photographs of the nerve cord of *Eudistylia vancouveri*.

FIGURE 1.—Longitudinal horizontal section through the nerve cord to show paired ganglia and commissures. Haematoxylin and eosin.

FIGURE 2.—Longitudinal vertical section through the cord showing a pair of ganglia and connectives. Haematoxylin and eosin.

FIGURE 3.—Transverse section through cord ganglia. Paired ganglia, commissure and giant axons shown. Heidenhain-azan.

FIGURE 4.—Transverse section through connectives of cord with giant axon above. Heidenhain-azan.

FIGURE 5.—Transverse section of ventro-lateral region of cord ganglion with nerve cells. Holmes's silver.

FIGURE 6.—Longitudinal horizontal section of cord and giant axon, with intersegmental constriction in the latter. Haematoxylin and eosin.

Legend: *BR*, giant axon branch(es); *CM*, commissure; *G*, cord ganglion (ganglia); *GA*, giant axon; *LC*, longitudinal connective; *LN*, peripheral nerve(s); *N*, nerve cord; *OC*, oesophageal connective; *SG*, supra-oesophageal ganglia; *SH*, giant axon sheath; *X*, intersegmental constriction in giant axon; *Y*, anastomosis of giant axons.

minal segments shrink to 15μ. These figures are mean figures of maximal horizontal and vertical diameters. Occasionally the giant axon reaches 500μ in one diameter in the anterior abdomen. Table I gives mean giant axon diameters for various regions of the body of three mature specimens for reference purposes, should this species prove suitable for physiological studies.

TABLE I

MEAN GIANT AXON DIAMETERS OF THREE MATURE SPECIMENS OF *Eudistylia vancouveri*

The length of the fixed animal (less crown) is given in the first column. Sample sections were cut at five equidistant levels along the length of the animals. The second column shows the distance of the first cut from the anterior prostomial edge Twenty-four measurements of maximal horizontal and vertical diameters were made in each region The means of each region are shown in the last five columns.

Specimen length, in.	*First cut, in*	*Giant axon diameters*				
		1	2	3	4	5
10	1	258.6	265	52.3	95.8	76.3
8¾	1¼	179.5	91.5	52.8	30	22.4
11¼	1¼	255.2	184.8	126.7	42.8	32.7

The diameter varies also in each segment. In sections cut longitudinally, the giant axons show slight and regular constrictions at segmental levels (Plate I, Fig. 6). This arrangement may be seen as well in the living animal if the dorsal body wall is slit open and pinned down, the gut removed, and the specimen studied under a binocular microscope. In such preparations the giant axons appear as clear channels somewhat constricted at septal levels, and bulging out in the middle of each segment.

Comparable segmental constrictions have been reported for the giant axons of other annelids but their significance is obscure. The condition is very apparent in the sabellid *Myxicola infundibulum* (Nicol, 1948). Stephenson (1912) found that the relatively large giant axons are greatly constricted in posterior segments of *Branchiura* (Oligochaeta) by the septa. In the capitellid *Mastobranchus*, which has myelinated giant axons, Friedländer (1889) stated that the axis cylinders are regularly constricted and the sheaths become very thin once in each segment ("segmental constrictions"). The arrangement in *Mastobranchus* is somewhat analogous to the constrictions in vertebrate myelinated nerve fibres, and in another invertebrate group, the Crustacae,' nodes of Ranvier have actually been described in *Leander* and *Palaemonetes* (Holmes, 1942; Nageotte, 1916). There is little resemblance, however, between such complexly organized nodes and the simple constrictions in *Eudistylia* and *Myxicola*, and it is probable that in the latter animals, regular alterations in axon diameter reflect segmental organization rather than signify peculiarity of functional mechanism.

Sheath of the giant axon

The giant axon is bounded by a collagenous sheath similar to and continuous with that enveloping the nerve cord. Ventrally, within the longitudinal connectives, the sheath forms a horizontal partition between the giant axon and the remainder of the cord. This horizontal partition is absent in the suboesophageal region and is interrupted within the ganglia of the cord (Plate II, Figs. 2-4). Between the collagenous sheath and the giant axon lies the axon sheath proper. This sheath varies from 4 to 44μ in thickness and consists of small supporting cells and fine fibres resembling the supporting tissue within the nerve cord. The cells and fibres are continuous with the latter tissue as sheaths investing the giant axon branches that descended into the neuropil. The cells and their nuclei are elongated in shape; cells and fibres tend to be arranged in a concentric reticulum about the giant axon. There is no stratification or internal differentiation within the sheath.

The giant axon sheath is unmyelinated by histological standards. Small pieces of the ventral body wall were immersed in osmo-picric acid fixative for periods varying from twenty-four to sixty hours; sections of this material failed to show any blackening of the sheath. Similarly, frozen sections stained with Sudan black showed no lipoid concentration about the giant axon.

Branches of the giant axon

Within or near the ganglia of the cord the giant axon gives off a number of branches ventrally into the neuropil (Plate II, Figs. 1, 3, 4). There are two to five branches to each ganglion. These branches are usually large at their origin from the giant axon, up to 32μ in diameter. The branches are continuous with the giant axon: sections fixed and stained in various ways and cut in different planes showed continuity of axoplasm at the junction.

The branches have a very complicated course in the neuropil. They regularly subdivide into smaller fibres which fan out among the nerve fibres and cells of the nerve cord. Frequently two branches which arise at nearly the same level anastomose with one another; occasionally successive branches join one another. It is apparent that these branches frequently form an anastomosing network which is connected with the giant axon at one or several loci.

In their ultimate ramifications the giant axon branches become very thin and in many places are difficult to distinguish from the ordinary small fibres of the nerve cord. It is necessary to emphasize the difficulties involved in tracing the smaller branches through the neuropil in serial sections because of the limitations which a solely neuro-histological

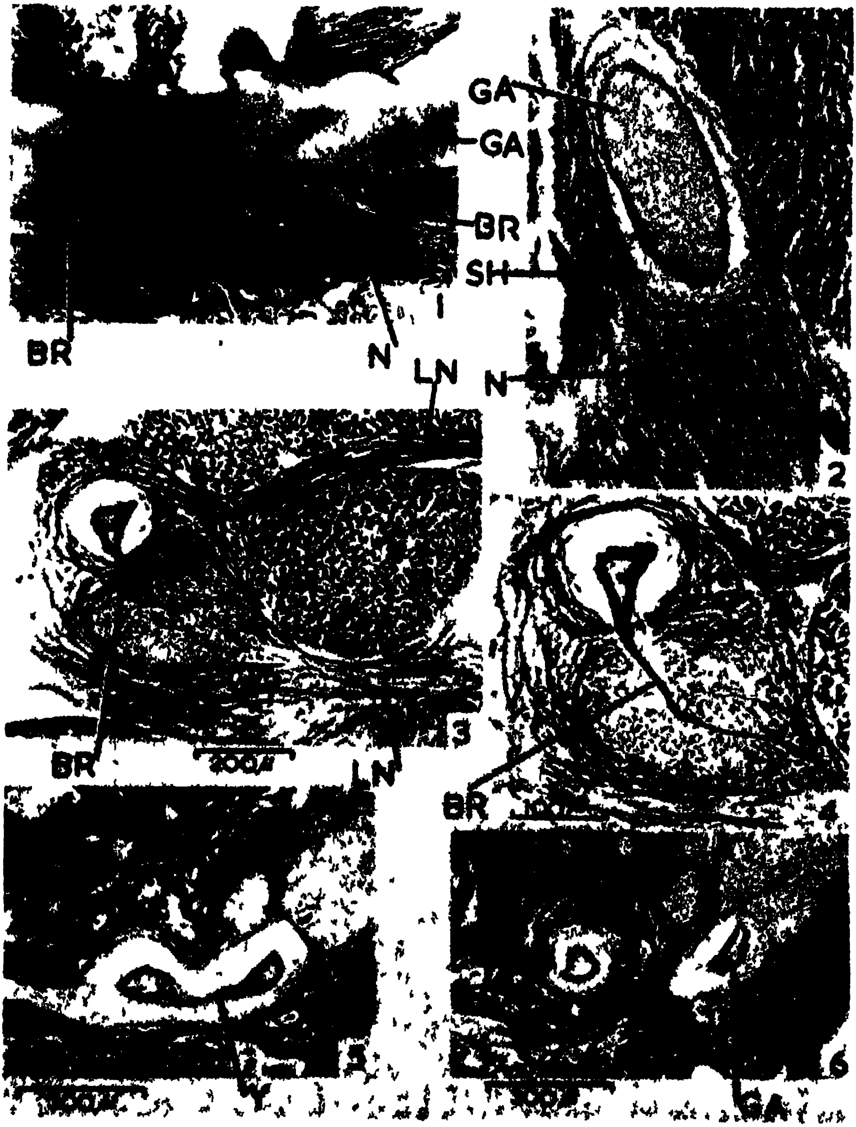


PLATE II

study imposes on attempts to elucidate the rôle of the giant axons in the functional economy of the entire animal. In order to formulate certain conclusions from the histological picture, several possibilities were investigated: do the branches of the giant axon (1) terminate in the neuropil in contact with other nerve fibres; (2) terminate in or on nerve cells; (3) enter peripheral nerves; (4) join the giant axons of the two sides of the nerve cord? These possibilities will be treated in turn.

1. Fine terminal fibres, arising from the subdivision of the giant axon branches, enter the cord and intermingle with the nerve fibres of the neuropil, and densely arranged nerve processes abut against the giant axon branches. These terminations are very small and it is not possible to analyse them in terms of special synaptic organelles, but the complexity and nature of their arrangement afford ample opportunity for establishing functional relations with other neurones entering and leaving the nerve cord.

2. Fibres from giant axon branches extend to nerve cells lying ventrally and ventro-laterally in the nerve cord; the fibres appear to be actual cell processes. The giant fibre cells resemble the other nerve cells in the cord and are not signalized by peculiarity of magnitude or form.

3. Repeated attempts to trace branches of the giant axon into peripheral nerves have been unsuccessful. It is probable that they do not enter nerve tracts leaving the cord.

4. Again, attempts to discover giant axon branches that enter the commissures between ganglia, and that connect together the two giant axons, were fruitless. The two giant axons appear to be unconnected throughout most of their length. The condition in the supra-oesophageal and suboesophageal ganglia is discussed below.

Occasionally one of the giant axons gives rise to a large subsidiary axon that runs independently a short distance through the nerve cord and anastomoses with the main axon again. The subsidiary axon may lie in a separate small nerve tract cut off from the main nerve cord by

---

PLATE II

Photographs of the nerve cord of *Eudistylia vancouveri.*

FIGURE 1.—Longitudinal vertical section of cord with giant axon branches. Masson's triple stain.

FIGURE 2.—Transverse section of cord and giant axon showing axon sheath. Holmes's silver.

FIGURES 3 and 4.—Transverse sections of nerve cord through ganglia. Giant axon branches and peripheral nerves shown. Holmes's silver.

FIGURES 5 and 6.—Transverse sections through suboesophageal region of the nerve cord. Two giant axons fusing together in Fig. 5, and separate in Fig. 6 prior to ascending the oesophageal connectives. Heidenhain-azan.

Legend as in Plate I.

a connective tissue sheath. Subdivision of giant axons and reunion of the portions appear to be common phenomena and have been reported for other annelid species: *Laonome* (Evenkamp, 1931), *Myxicola* (Nicol, 1948), *Pomatoceros* (Thomas, 1940), *Neanthes* (Hamaker, 1898),

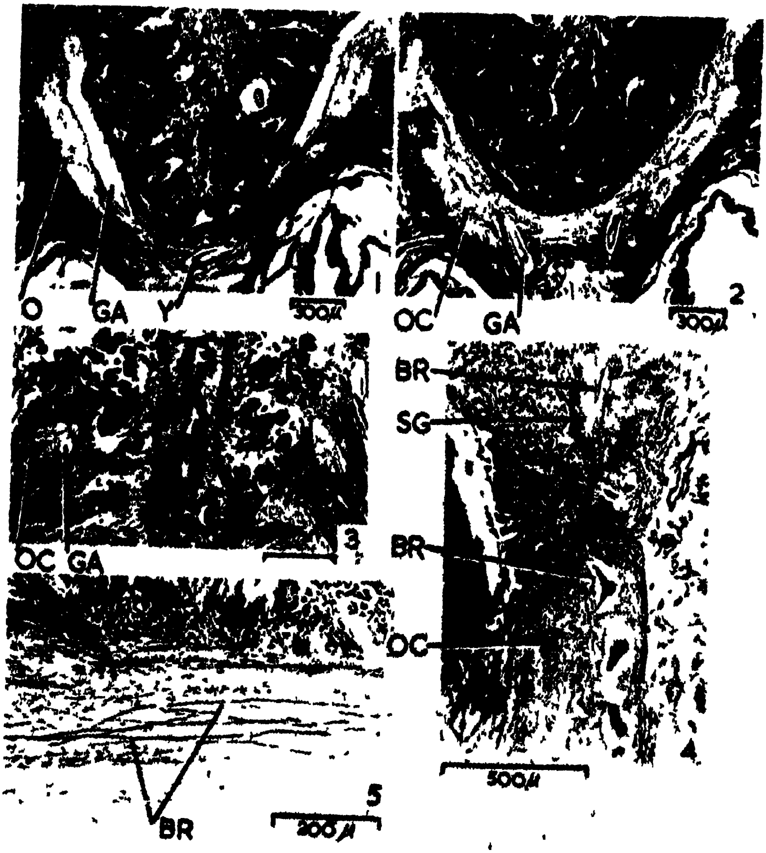


PLATE III

Photographs of anterior c.n.s. of *Eudistylia vancouveri*

FIGURES 1, 2, and 3. Longitudinal horizontal sections through oesophageal connectives. Fig. 1 shows anastomosis of two giant axons. Heidenhain-azan.

FIGURE 4. Junction of oesophageal connective with lobe of supra-oesophageal ganglia. Branches and subdivision of giant axon shown. Transverse section. Holmes's silver.

FIGURE 5. Transverse section through dorsal commissure of supra-oesophageal ganglia. Branches of the giant axons from either side meet in the mid-dorsal plane. Holmes's silver.

Legend as in Plate I.

Lumbricus (Stough, 1926). Such supernumerary giant fibres are the exception rather than the rule and probably represent developmental variability.

Giant axons in the head

The giant axons in the suboesophageal region have an arrangement different from more posterior segments. The suboesophageal ganglia are considerably flattened and the giant axons lie within the neuropil of the cords. In about half the specimens examined the two axons fuse with one another by an anastomosis in the suboesophageal ganglia (Plate II, Figs. 5, 6; Plate III, Fig. 1). The two axons then extend forwards beneath the brain, and send branches into the oesophageal connectives. The branches divide repeatedly, forming a group of five to ten fine fibres which ascend into the supra-oesophageal ganglia (Plate III, Figs. 1-4). Some at least of these fibres terminate in small nerve cells lying laterally, near the origin of the branchial nerve tracts, and medially, in the dorsal commissure between the lobes. Giant axon fibres from either side of the animal meet in the dorsal commissure. The fibres are small in this region, 4μ or less in diameter, and are difficult to disentangle. They approach each other and run side by side, apparently without actual fusion. The arrangement is such as to permit axonic contact and functional synapsis even if neuroplasmic discontinuity should exist (Plate III, Fig. 5).

Axoplasm of the giant axons

The giant axons are continuous structures throughout their length, and there are no signs of segmental septa such as occur in *Nereis* and *Lumbricus* (Stough, 1926). This point was checked carefully in sections fixed in Flemming's solution and cut longitudinally (cf. Young, 1936). In this respect the giant axons of *Eudistylia* resemble those of *Myxicola* (Nicol, 1948).

In the living animal the giant axons appear as translucent tubes. They do not lend themselves readily to careful dissection for high power observation. Short stretches of axoplasm can be squeezed out and recovered from the nerve cord and this axoplasm has a thin gelatinous consistency, resembling that of the sabellid, *Myxicola* (Nicol, 1948). In fixed sections, the axoplasm contains fine lines and whirls similar to those seen in *Sabella* (Cunningham, 1887) and *Myxicola* (Nicol, 1948). Such lines probably reflect ultrastructural organization in the giant axons (cf. Young, 1937).

Neuronal fusion in the giant axons

The fact that the two giant axons of *Eudistylia* occasionally, but not always, fuse together in the suboesophageal ganglia suggests that they are multicellular structures. It has already been postulated that they arise from nerve cells in the supra-oesophageal ganglia, and are united with nerve cells throughout their length in the ganglia of the cord. Since the ultimate ramifications of the giant axons are very slender, this hypothetical arrangement requires ancillary evidence. In order to discover whether these axons are multicellular, the nerve cords of fifteen animals were cut at the junction of thorax and abdomen and the animals sacrificed at periods ranging from eleven to twenty-one days. The animals were kept in jars of aerated sea water at room temperature (15°-20° C.); the water was changed daily. Mortality was heavy, but two animals were obtained viable at twenty-one days and others at shorter intervals after operation. Thoracic and abdominal nerve cords were prepared as histological sections. There was no degeneration of the giant axons in the specimens examined; they remained intact on both sides of the lesion up to twenty-one days after section.

Although the majority of work on nerve degeneration has been done on mammals, some data are available for poikilothermous animals. In the teleost, *Uranoscopus*, severing the sympathetic trunks and the oculomotor nerve produced degeneration of fibres in the peripheral stumps six to eighteen days after injury (Young, 1931). Holmes (1942) found true "Wallerian" degeneration of nerve fibres in the prawn eleven days after section of the circumoesophageal connective, while the median giant fibres, presumably syncytial, were not degenerate fourteen days after section. Sereni and Young (1932) have studied the processes of degeneration and regeneration of nerve fibres in cephalopods. They found that the course of nervous degeneration was rapid in these animals and that axons cut off from their cell bodies were fragmenting after one day (summer temperatures, Naples). The giant axon of *Myxicola* fails to degenerate up to sixteen days after transection and is syncytial (Nicol and Young, 1946; Nicol, 1948). If the giant axons of *Eudistylia* are unicellular, secondary degeneration should occur on one side of the cut by twenty-one days. Failure to degenerate on either side of the lesion and absence of large cells commensurate with the size of the axons render it probable, therefore, that these structures are multicellular and that they unite with small nerve cells in thorax and abdomen.

DISCUSSION AND CONCLUSIONS

The giant fibres of *Eudistylia* are a pair of very large axons that extend from the supra-oesophageal ganglia to the end of the nerve cord. Since they are continuous structures they provide through-conducting pathways throughout the length of the c.n.s. The pattern of arrangement in this species is similar to that found in some other sabellids with ladder-like nerve cords. *Laonome, Euchone, Sabella, Spirographis,* and *Branchiomma* have paired giant axons of large size extending through the nerve cord and, in the last four species at least, the giant axons enter the oesophageal connectives and proceed into the supra-oesophageal ganglia (Claparède, 1873; McIntosh, 1877; Cunningham, 1887; Brunotte, 1888; Evenkamp, 1931). Claparède found connections between the two giant axons in the thoracic commissures of *Spirographis.* In *Myxicola* the two giant axons arise from a pair of cells in the supra-oesophageal ganglia, are connected together in the thoracic commissures, and fuse to form a single fibre in the abdomen. Although information is still incomplete, the arrangement in *Eudistylia,* compared with other species studied, allows the following picture to be postulated of giant axons in the Sabellidae. There are two giant axons which extend uninterrupted throughout the nerve cord. The axons arise from nerve cells in the supra-oesophageal ganglia where they meet and decussate without fusion. When the nerve cord is ladder-like, one axon lies in each half of the cord (*Sabella, et al.*); when a single cord is present, the axons unite to form a single median giant fibre (*Myxicola*). In any case the axons may be joined by transverse anastomoses in the thoracic segments. The fibres are connected to nerve cells throughout the nerve cord. They lack a well-developed myelin sheath.

The existence of multicellular neurones in various invertebrates is now well-established. In the prawn, long-distance giant axons are probably syncytial and the large segmental motor axons in this animal are bicellular (Holmes, 1942; Johnson, 1923). The giant axons in the stellar nerves of the squid and cuttlefish arise from many nerve cells (Young, 1939). In the Annelida, multicellular giant axons have been described in many species: *Lumbricus* (Friedländer, 1888; Stough, 1926), *Criodrilus* (Honig, 1910), *Mastobranchus* (Friedländer, 1889), *Arenicola* (Gamble and Ashworth, 1900), *Clymenella* and *Clymene* (Lewis, 1898), *Myxicola* (Nicol, 1948), and bicellular axons in *Neanthes* (Hamaker, 1898) and *Euthalenessa* (=*Sthenelais*) (Rohde, 1887). In certain animals it has been shown that neuronal fusion of giant axons permits widespread distribution of efferent impulses throughout these nervous pathways, for example, the first order giant axons of *Loligo* (Young, 1939) and the lateral giant fibres of *Lumbricus* (Stough, 1930; Rushton, 1945). With the assumption of functional contact in the supra-oesophageal ganglia of *Eudistylia* and occasional fusion in the suboesophageal ganglia, the two giant axons of this species presumably would con-duct as a unit. Moreover, the giant axons of *Eudistylia* and *Myxicola* are so large that their numerous small nerve cells should have a trophic significance in maintaining such large volumes of protoplasm. In this regard it is interesting that in *Halla*, which has unicellular giant axons, the nerve cells themselves are giant in size.

Intersegmental giant axons in annelids form through-conducting nervous pathways for quick shortening movements ("startle reactions"). This relationship has been well established for the earthworm (Stough, 1930, and others) and more recently for various polychaetes (Bullock, 1945, 1948; Nicol, 1948a). *Eudistylia* shows a quick withdrawal reflex on stimulation and jerks back quickly into its tube. In its behaviour and in the arrangement of the giant axons *Eudistylia* resembles the allied form, *Myxicola*, which has been studied more intensively. In both animals the extensive branching of the giant axons in the neuropil of the c.n.s. permits ample opportunity for frequent contact with afferent pathways throughout the length of the animal; and the large axonic diameters determine rapid conduction in these fibres. In *Myxicola* the giant axon itself is the efferent path since large motor branches, in protoplasmic continuity with it, enter the peripheral nerves. Such branches are not apparent in *Eudistylia* and it is probable that the giant axons of this species, although efferent pathways, do not directly innervate the longitudinal musculature involved in the withdrawal reflex.

SUMMARY

1. The sabellid worm *Eudistylia vancouveri* possesses a pair of giant axons which extend throughout the length of the ladder-like nerve cord. These axons send branches through the oesophageal connectives into the supra-oesophageal ganglia. In mature specimens they are about 250μ in diameter in the thorax and taper off to 15μ in posterior segments.

2. Axoplasm is continuous throughout the length of the fibres. Expressed axoplasm has a gelatinous consistency.

3. The giant axons show slight intersegmental constrictions in diameter. They are invested by an outer collagenous and an inner neuroglia-like sheath which is not myelinated.

4. Branches from the two giant axons meet in the supra-oesophageal ganglia, apparently without fusion, and terminate in nerve cells. In some individuals the two giant axons anastomose in the suboesophageal portion of the nerve cord.

5. Several branches of the giant axons extend into the neuropil in each ganglion. Here they subdivide, pass to nerve cells, and meet other neurons.

6. Evidence is presented that these axons are syncytial; they show connections with many nerve cells in the cord ganglia; there are no giant nerve cells commensurate with the size of the axons; cutting the giant axons at thorax-abdominal level fails to produce degeneration in the fibres on either side of the lesion up to twenty-one days after injury.

7. Similarities between the giant axons of *Eudistylia* and other sabellids are described, and their arrangements and disposition as efferent pathways in the "startle" reflex are considered.

REFERENCES

ASHWORTH, J. H. (1909). The giant nerve cells and fibres of Halla parthenopeia. Proc. Roy. Soc. Lond., **B200**: 427-521.

BERKELEY, E. and BERKELEY, C. (1942). North Pacific Polychaeta, chiefly from the west coast of Vancouver Island, Alaska and Bering Sea. Canad. J. Res., **D20**: 183-208.

BRUNOTTE, C. (1888). Recherches anatomiques sur une espèce du genre Branchiomma. Trav. Sta. Zool. Cette, pp. 77. Quoted by Ashworth, 1909.

BULLOCK, T. H. (1945). Organization of the giant nerve fiber system in Neanthes virens. Biol. Bull., **89**: 185-6 (Abstract).

———(1948). Physiological mapping of giant nerve fiber systems in polychaete annelids. Physiologia Comparata et Oecologia, **1**: 1-14.

BUSH, K. J. (1910). Tubicolous annelids of tribes Sabellides and Serpulides from the Pacific Ocean. Harriman Alaska Series, Smithsonian Institute, **XII**: 167-355.

CLAPARÈDE, E. (1873). Recherches sur la structure des annélides sédentaires. Mém. Soc. Phys. Genève, **22**: 1-200.

CUNNINGHAM, J. T. (1887). On some points in the anatomy of Polychaeta. Quart. J. Micr. Sci., **28**: 239-78.

EVENKAMP, H. (1931). Morphologie, Histologie und Biologie der Sabellidenspecies Laonome Kroyeri Malmgr. und Euchone papillosa M. Sars. Zool. Jahrb. Abt. Anat. Ont., **53**: 405-534.

EWER, D. W. (1941). The blood systems of Sabella and Spirographis. Quart. J. Micr. Sci., **82**: 587-620.

FRIEDLÄNDER, B. (1888). Beiträge zur Kenntnis des Centralnervensystems von Lumbricus. Zeit. wiss. Zool., **47**: 47-84.

———(1889). Über die markhaltigen Nervenfasern und Neurochorde der Crustaceen und Anneliden. Mitt. Zool. Sta. Neapel, **9**: 205-65.

GAMBLE, F. W. and Ashworth, J. H. (1900). The anatomy and classification of the Arenicolidae, with some observations on their post-larval stages. Quart. J. Micr. Sci., **43**: 419-569.

HAMAKER, J. I. (1898). The nervous system of Nereis virens Sars: A study in comparative neurology. Bull. Mus. Comp. Zoöl. Harv., **32**: 87-124.

HARTMAN, O. (1938). Annotated list of the types of polychaetous annelids in the Museum of Comparative Zoölogy. Bull. Mus. Comp. Zoöl. Harv., **85**: 1-31.

———(1942). The identity of some marine annelid worms in the United States National Museum. Proc. U.S. Nat. Mus., **92**: 101-40.

HAVET, M. J. (1916). Relations de la névroglie avec l'appareil vasculaire chez les invertébrés. C. R. Acad. Sci., **162**: 568-70.

Holmes, W. (1942). The giant myelinated nerve fibres of the prawn. Phil. Trans. Roy. Soc. Lond., **B231**: 293-311.

—— (1947). Axon stain; in Recent advances in clinical pathology, ed. S. C. Dyke. Churchill, London.

Hönig, J. (1910). Die Neurochorde des Criodrilus lacuum. Hoffmstr. Arb. Zool. Inst. Wien, **18**: 257-82.

Johnson, G. E. (1924). Giant nerve fibres in crustaceans, with special reference to Cambarus and Palaemonetes. J. Comp. Neur., **36**: 323-73.

Lewis, M. (1898). Studies on the central and peripheral nervous system of two polychaete annelids. Proc. Amer. Acad. Arts Sci., **33**: 225-68.

McIntosh, W. C. (1877). On the arrangement and relations of the great nerve-cords in the marine annelids. Proc. Roy. Soc. Edin., **9**: 372-81 (Abstract).

Meyer, E. (1887). Studien über den Körperbau der Anneliden. Mitt. Zool. Sta. Neapel, **7**: 592-741.

—— (1888). *Ibid.*, **8**: 462-662.

Nageotte, J. (1916). Note sur les fibres à myeline et sur les étranglements de Ranvier chez certains crustacés. C. R. Soc. Biol., **79**: 259-63.

Nicol, J. A. C. (1948). The giant nerve fibres in the central nervous system of Myxicola (Polychaeta, Sabellidae). Quart. J. Micr. Sci., **89**: 1-45.

—(1948a). The function of the giant axon of Myxicola infundibulum Montagu. Canad. J. Res., **D26**: 212-22.

Nicol, J. A. C. and Young, J. Z. (1946). Giant nerve fibre of Myxicola infundibulum (Grube). Nature, **158**: 167.

O'Donoghue, C. H. (1923). A note on the polychaetous annelid Eudistylia gigantea Bush. Contr. to Can. Biol., **1** (n.s.): 443-53.

Pantin, C. F. A. (1946). Notes on microscopical technique for zoologists. Cambridge, at the University Press.

Rhode, E. (1887). Histologische Untersuchungen über das Nervensystem der Polychaeten. Zool. Beitr., **2**: 1-81.

Rushton, W. A. H. (1945). Action potentials from the isolated nerve cord of the earthworm. Proc. Roy. Soc. Lond., **B132**: 423-37.

Sereni, S. and Young, J. Z. (1932). Nervous degeneration and regeneration in cephalopods. Pubbl. Staz. Zool. Nap., **12**: 173-208.

Stephenson, J. (1912). On Branchiura sowerbyi Beddard, and on a new species of Limnodrilus with distinctive characters Trans. Roy. Soc. Edin., **48**: 285-303.

Stough, H. B. (1926). Giant nerve fibers of the earthworm. J. Comp. Neur., **40**: 409-63.

——(1930). Polarization of the giant nerve fibers of the earthworm. J. Comp. Neur., **50**: 217-29.

Thomas, J. G. (1940). Pomatoceros, Sabella and Amphitrite. L.M.B.C. Memoir XXXIII. Liverpool, University Press.

Young, J. Z. (1931). The pupillary mechanism of the teleostean fish Uranoscopus scaber. Proc. Roy. Soc. Lond., **B107**: 464-85.

—— (1935). Osmotic pressure of fixing solutions. Nature, **135**: 823-5.

——(1936). Structure of nerve fibres and synapses in some invertebrates. Cold Spring Harb. Symp. Quant. Biol., **4**: 1-6.

——(1937). The physical and chemical properties of nerve fibres and the nature of synaptic contacts. Trans. Faraday Soc., **33**: 1035-40.

——(1939). Fused neurons and synaptic contacts in the giant nerve fibres of cephalopods. Phil. Trans. Roy. Soc. Lond., **B229**: 465-503.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

A Discussion of the Mortality in Pink Salmon (*Oncorhynchus gorbuscha*) during Their Period of Marine Life

By A. L. PRITCHARD

Presented by R. E FOERSTER, F.R.S.C.

ALTHOUGH considerable information is now available on the losses occurring during the freshwater phase of the life of certain Pacific salmon, genus *Oncorhynchus* (Barnaby, 1944; Foerster, 1936; Pritchard, 1940, 1947, and 1948), comparatively little of either a qualitative or quantitative nature has been presented concerning the reduction in numbers during the years of life which are spent in the ocean environment. The obvious reason for such a situation appears to be that investigators have not yet been able to gather evidence which they consider definite and conclusive. It seems, however, that such material as is available about the pink salmon (*O. gorbuscha*) should be submitted herein if only to put it and the opinions resulting therefrom on record as a basis for further investigation.

METHODS

In a previous paper (Pritchard, 1948) the writer described in detail an experiment designed to investigate the freshwater phase of the natural propagation of pink salmon and to reveal the losses therein. This work, carried out at McClinton Creek, Masset Inlet, British Columbia, consisted mainly of three activities: (1) the counting of the adult salmon proceeding upstream to spawn; (2) the calculation of the potential egg deposition (for this was employed the average number of eggs per individual obtained by actual count of a random sample of females and the total number of females); and (3) the enumeration of the number of fry resulting from each seeding as they migrated to sea during the following spring. In order to extend the range of this experiment and to use the counts and calculations in a discussion of the losses occurring in the ocean phase of the life between the time when the fry left the stream and returned as adults to spawn, it was essential to determine: (*a*) at what age the species matured or, in other words, to discover of which fry migrations certain adults were the progeny, and (*b*) whether the pink salmon returned unerringly to the stream in which they originated. With this information the number of spawning adults which resulted from a known egg deposition and fry escapement would be revealed.

The collection of these basic data began with the spawning run of 1930, and was continued for each such migration thereafter up to that of 1942. Each spring from 1931 to 1941 the resulting fry were enumerated.

DISCUSSION OF THE PROBLEM

Age at maturity

Using the method of marking (by the removal of certain combinations of fins with nail clippers) it was established early (Pritchard, 1939) that *all* pink salmon matured in the autumn of their second year. Thus, each spawning migration at McClinton Creek was the result of a similar escapement two years previously.

Homing tendency

Many tests have been made to discover whether pink salmon return to spawn in the streams from which they originated. The author (Pritchard, 1939), after reviewing earlier experiments, including those carried out by Davidson (1934), reached the conclusion "that the majority of the fish return to the parent stream but that isolated individuals may wander almost four hundred miles from the original area." This small number of wanderers was considered economically insignificant.

A later marking experiment conducted in the spring of 1939 (Pritchard, 1941), however, confirmed the fact that there was always a discrepancy between the percentage returns of marked and unmarked salmon to McClinton Creek. The unmarked proportion was always consistently lower. This condition appeared to be assignable to one or all of three causes, namely (1) there was a relatively greater mortality of marked pinks as a result of the operation (*differential mortality*), (2) some of the fins might have regenerated with the result that the marked salmon could not be differentiated from the unmarked (*regeneration*), and (3) there might have been wandering of marked individuals to other creeks coupled with an influx of unmarked fish to McClinton (*wandering*).

The first two of the above-mentioned causes, *differential mortality* and *regeneration*, have been discussed at length in another paper (Pritchard, 1939). In a number of experiments where marked fry and normal controls were held under similar conditions in ponds for some months, little evidence was obtained of any adverse effect from marking. No differential mortality was demonstrated. If the fins were not completely removed, there was danger of regeneration, but, with experienced operators, this possibility was kept at a minimum. While both causes undoubtedly had an effect, it did not appear on the basis of the data available at the time, that either singly would be sufficient to account for the moderately large difference, or that the combined effect of both would explain all the discrepancy.

Until the season of 1940, it was impossible to get any information on *wandering* by thoroughly checking for marked pinks other streams tributary to Masset Inlet or elsewhere. During that year, however, four major rivers were visited to estimate the spawning escapement, to examine a random sample of the population, and to determine the proportion of marked fish therein. The data gathered are presented in Table 1.

The best estimate of the proportion of marked to unmarked pink salmon in these four "outside" rivers should be given by the percentage which the number of marked fish discovered constitutes of the number examined in finding them. On the other hand, it is essential to set some degree of confidence concerning the limits between which these values can lie. For this purpose, the method presented by Ricker (1937) has been employed. Adopting the 0.99 confidence coefficient, the possible upper and lower limits and best estimates of market fish have been calculated for the escapements.

TABLE I

Best estimates and possible lower and upper limits in numbers of marked fish in the Yakoun, Mammon, Deltamen and Deenan Rivers in 1940 calculated from the table of confidence limits (Ricker 1937, p. 354) for a confidence coefficient of 0.99 and on the basis of the size of the sample in relation to the estimate of total escapement.

River	*Estimate of total escapement*	*Number examined*	*Number of marked pinks discovered*	*Marks in estimated escapement*		
				Lower limit	Best estimate	Upper limit
Yakoun	500,000	1,847	0	0	0	1,435
Mammon	100,000	3,400	1	0	30	218
Deltamen	25,000	400	1	0	63	463
Deenan	10,000	275	0	0	0	193
TOTAL	635,000	5,922	2			

Table 1 shows that in the samples examined in the four specified rivers (total 5,922), two marked fish were discovered. On the basis of the proportion between the numbers inspected and those actually found, and considering the size of the estimated escapements in comparison with that of the sample handled in each, the best estimate of marked fish in the particular 635,000 pink salmon is probably 93. On the other hand, it is very unlikely that there might have been no marked individuals or more than 2,309.

In this connection it is interesting to caluculate just what the situation would have been in the sample inspected from each river if the proportion had been the same as was found in McClinton Creek itself in 1940, namely 50 in 2,274. These figures are presented in Table II.

TABLE II

Number of marked fish most likely to be encountered and lowest number to be expected in samples examined in the Yakoun, Mammon, Deltamen and Deenan Rivers in 1940 as calculated from the table of confidence limits (Ricker 1937, p. 354), for a confidence coefficient of 0.99 and on the basis of the proportion of marked fish found in McClinton Creek during the same year.

River	*Number examined*	Actually found	*Number of marked pinks* Most likely on basis McClinton ratio	Lowest number expected on basis McClinton ratio
Yakoun	1,847	0	41	26
Mammon	3,400	1	75	55
Deltamen	400	1	9	3
Deenan	275	0	6	2
Total	5,922		131	86

From Table II it can be seen that the number of marked pinks most likely to be encountered on the basis of the McClinton Creek ratio is 131, and that it is unlikely that on the same basis fewer than 86 would be found. Comparison of these numbers with the two actually discovered *indicates beyond reasonable question that the great majority of pink salmon do in fact return to the parent stream.*

This conclusion is further confirmed by a study of the concentration of the marked fish in the fishery in relation to that on the spawning beds in the experimental area. Again using the year 1940 as an example, in a catch of approximately 175,000 taken on the southeastern side of Masset Inlet, off Cub Island, Kumdis Island, Port Clements, and Ferguson Bay, where it is known that fish from almost all the rivers are involved, 142 marked McClinton pinks were located or approximately 0.8 individuals in a thousand. In the fishery at McClinton Bay, 92 marks were discovered in 6,000 or 15.3 per thousand. The return in McClinton Creek itself was but little higher: 781 in 35,521 or about 22 marks per thousand.

There still remains the possibility that wandering may have taken place to streams other than those in Masset Inlet. On the basis of data available, however, the likelihood must be considered as small. From all the McClinton marking experiments, no recoveries were made on spawning streams elsewhere. McClinton fish were captured in "outside" fisheries but, since they were travelling, they might have returned to the parent stream if they had not been caught. Further, Davidson (1934) concludes "that the pink salmon composing the runs in streams that are more or less isolated from other pink-salmon streams may show little or no tendency towards straying, whereas the pink salmon in streams flowing into bays and in close proximity to other pink-salmon streams may stray more or less into the neighbouring streams."

From the above discussion, it would certainly appear that *wandering* will not account in itself for the discrepancy in the proportionate returns of marked and unmarked pink salmon. Likewise neither *differential mortality* nor *regeneration* of fins can be considered as sufficient explanation. The effect of all three causes must be recognized, and further investigation is indicated to set the contribution of each.

From the point of view of the discussion of ocean mortality, the important fact to consider is that the comparison of the figures for the percentage return of adults from one cycle to another in order to determine the *absolute* and *definite* difference in ocean mortality might be inaccurate. On the other hand, since there is every indication that the return to the parent stream constitutes by far the greater proportion of the population which originated there and survived the ocean life, it is permissible to make broad comparisons to indicate the general trend without placing too much emphasis on the actual numbers involved.

RESULTS

A complete record of the counts for the duration of the experiment are submitted in Table III.

Variation in ocean mortality

Even after consideration of all the limitations just discussed, the conclusion may safely be drawn from Table III that there is a great difference from cycle to cycle in the percentage survival of spawning adults determined on the basis of the numbers of fry migrants or spawning individuals in the previous run. This variation in the freshwater phase (fry/eggs) may be as large as 3.5 times. The mortality ranges from 76.2 to 93.1 per cent of the potential. From the remaining 6.9 to 23.8 per cent, the minority which enter the ocean phase, variation in percentage survival may be as great as 23 times and the mortality 93.25 to 99.71 per cent.

If one could consider an equal number of potential salmon entering the freshwater phase as eggs or the ocean phase as fry, it would be ex-

TABLE III

Propagation counts at McClinton Creek for the six life cycles of pink salmon between 1930 and 1942 with the percentage survival in each phase and in complete life

	1930	*1932*	*1934*	*1936*	*1938*	*1940*	*1942*
Escapement	66,153	15,600	155,196	52,312	10,577	35,521	36,893
Egg deposition (Millions)	50.8	13.2	139.5	53.2	8.5	26.6	
Numbers of fry (Millions)	5.4	2.3	12.6	3.7	2.0	5.0	
Percentage survival							
Freshwater phase							
Fry/eggs	10.6	17.4	9.0	6.9	23.8	19.0	
Ocean phase							
Adults/fry	29	6.75	.42	.29	1.78	.74	
Complete life							
Adults/adults	24.06	994.84	33.71	20.22	335.83	103.96	

pected that the percentage survival at the end of the former (1-3.5 times) would vary less than that at the end of the latter (1-23 times). The *percentage* mortality due to all causes would apparently be greater in the ocean although the *absolute* mortality would be less.

Causes of variation in ocean mortality

Amongst the more important agents which might influence the variation in ocean mortality might be listed the following:

1. The variation in predation on the fish in the sea resulting from changes in the numbers, distribution and feeding behaviour of predators in different years.

2. The variation from year to year in climatic and hydrographic conditions which might affect differently (*a*) the growth and distribution of the food organisms, and (*b*) the behaviour and movements of the salmon, and thus the distribution of the fishery.

Variation in predation

No extensive and reliable information is available yet on the numbers, distribution, and behaviour of the *ocean predators* of pink salmon. In the absence of such specific knowledge, the possibility must be recognized that they, like other closely related animals, may be subject to fluctuations in abundance and changes in behaviour. Such deviations would undoubtedly have the effect of increasing or decreasing the amount of predation.

Variation in climate and hydrographic conditions

Meteorology and *ocean hydrography* or *oceanography* in their modern aspects must be considered relatively young sciences. It is only in recent years that there seems to have arisen the incentive to collect the massive amount of data necessary to determine the effect of climate on conditions in the ocean. Still more recent is the effort to relate either set of information with the behaviour of marine organisms. Sufficient has been accomplished to indicate that changes in temperature, tides, currents, etc. may not only influence the growth of *salmon food organisms,* but may also remove them to other areas. This would have the effect of changing the amount of food available and perhaps altering the route of migration of the fish themselves. The interrelationships in this regard are very complex and give ample opportunity for variation.

There is already certain evidence which indicates that the *route of migration of pink salmon may vary* from year to year as a result of changes in climatic conditions. In some seasons large numbers of these fish are taken by trollers at Langara Island and along the northern coast of Graham Island before the main concentration appears in Masset Inlet. In other years very few are encountered in these outside districts. In addition, in 1940 and 1942, further information of a pertinent nature was gained from an examiner stationed at Waterfall cannery on the west coast of Prince of Wales Island in southeastern Alaska to check the catches for pink salmon originally marked at McClinton Creek in Masset Inlet. In 1940, from the marking of 178,629 fry, thirty scarred individuals were located among approximately two million individuals seined in the vicinity of Noyes, Baker, and Dall Islands relatively close by. In 1942, from a marking of 198,297 fry, only two marked pinks were discovered in approximately one and one-half million from the same areas. While it is known that this type of gear could conceivably be limited to certain areas in one year by weather conditions, the report of the examiner gives no reason to believe that such was the case in the seasons under discussion. It has been strongly suggested that predominately southeasterly winds in one year and predominately northwesterly in the other may have caused the difference. While the data available would seem to support this hypothesis, they are not sufficiently comprehensive or reliable to either definitely confirm or deny it.

That changes in the *distribution and intensity of the fishery* could serve to cause some variation in the return to a creek such as McClinton, might be inferred from the example given above for southeastern Alaska. There is no doubt that the areas exploited and the effort expended will differ from year to year depending on such things as climatic conditions, demand for fish, etc. As a result the percentage survival will vary. In Masset Inlet itself, the fishery has a very definite bearing on the numbers which may reach any river to spawn. The major effort is usually concentrated off the mouth of the Yakoun River in the southeastern corner near Port Clements. It has been demonstrated that McClinton pinks are taken here, although in much smaller relative numbers than in McClinton Bay. In some seasons, when heavy westerly winds prevail and drive the waves on to the southern shore, the fleet is forced to seek the heads of the long bays for protection. For this reason the McClinton Bay catch has varied from a few thousand in one year to almost two hundred thousand in another. It is evident that the spawning population was more heavily drained in the latter case.

In spite of the possibility of variation which may result from all the changing factors mentioned, it is interesting to find from Table III that in cycles when the efficiency of hatch (percentage survival of fry from eggs) is high, i.e. 1932, 1938, and 1940, the percentage survival in adults on any basis is also high. Conversely, in years when the efficiency of hatch is low, i.e. 1930, 1934, and 1936, the percentage adult survival is also low. That the favourable or unfavourable level of survival shown when the fry leave the river seems to persist throughout the ocean phase of the life is indicated particularly by the relatively low return in adults on the basis of fry migrants in the seasons of poor hatch as compared with the relatively high figures for seasons of good hatch.

In a previous paper (Pritchard, 1948), it was stated that "within certain limits - and under reasonably uniform climatic conditions, the smaller seeding of pink salmon will generally provide the greater efficiency of hatch." The exact cause or causes for this are not definitely known but they are assumed to be bound up with the reaction between the fish and its environment—both physical and biological. It was suggested that when the number of spawning salmon was reduced, certain limiting factors might become less intense in their action. To the tendency toward building up the population, the term "resilience" was assigned. A negative correlation between fry number and the adult/fry ratio suggests that the tendency may also be active in the ocean period, but the lack of significance of this correlation precludes the definite statement that its existence is proven.

It should be evident from the foregoing presentation that further studies of the ocean phase of the life history of pink salmon are important in order to elucidate the effect of the factors which may cause variation in survival. Perhaps little can be done to control such factors toward increasing the run, but if their influence was clearly known, predictions might be possible which would allow the industries utilizing the resource to anticipate their needs and organize their operations on a more efficient and effective basis.

REFERENCES

BARNABY, J. T. (1944). Fluctuations in abundance of red salmon, *Oncorhynchus nerka* (Walbaum), of the Karluk River, Alaska. Bull. U.S. Fish and Wildlife Serv. 50, Fish. Bull. 39: 237-95.

DAVIDSON, F. A. (1934). The homing instinct and age at maturity of pink salmon (*Oncorhynchus gorbuscha*). Bull. U.S. Bur. Fish. 48, Bull. 15: 27-39.

FOERSTER, R. E. (1936). Sockeye salmon propagation in British Columbia. Biol. Board of Canada, Bull. 53: 1-16.

PRITCHARD, A. L. (1939). Homing tendency and age of maturity of pink salmon (*Oncorhynchus gorbuscha*) in British Columbia. J. Fish. Res. Board of Canada **4**: 233-51.

(1940). A study of the natural propagation of the pink salmon, *Oncorhynchus gorbuscha*, in British Columbia. Trans. Amer. Fish. Soc. **69** (1939): 237-9.

— - (1941). The recovery of marked Masset Inlet pink salmon during the season of 1940. Fish. Res. Board of Canada, Prog. Rept. (Pac.) **48**: 13-17.

———(1947). Efficiency of natural propagation of Pacific salmon. Canad. Fish. Cult. **1**: 22-6.

———(1948). Efficiency of natural propagation of the pink salmon (*Oncorhynchus gorbuscha*) in McClinton Creek, B.C. J. Fish. Res. Board of Canada **7**: 224-36.

RICKER, W. E. (1937). The concept of confidence or fiducial limits applied to the Poisson frequency distribution. J. Amer. Stat. Assoc. **32**: 349-56.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

The Efficacy of Catch Limitations in Regulating the British Columbia Herring Fishery

By ALBERT L. TESTER

Presented by R. E. FOERSTER, F.R.S.C.

INTRODUCTION

A purse seine fishery for herring (*Clupea pallasii*) has been active along the coast of British Columbia for over thirty-five years. Following an early period of rapid development the fishery has been pursued with varying effort which, in general, has tended to increase in recent years. As in the case of most marine fisheries, there have been fluctuations in the catch which have been variously interpreted as either natural or induced. Upward fluctuations, good fishing seasons, have been accepted with satisfaction. Downward fluctuations, poor fishing seasons, have been viewed with some alarm by administrators whose responsibility it is to avoid a progressive decrease in abundance due to overfishing, by salmon trollers and other fishermen who believe that an abundance of herring is essential in order to maintain the supply of spring salmon and other fish which feed on herring, and even by the herring fishermen themselves who are concerned with their future livelihood.

Fear of depletion has stimulated the enforcement of regulations restricting fishing effort. Of these, perhaps the most useful and effective is the closed season during the spawning period which has been in force for many years, and which prevents fishing at a time when the herring are not only in poor condition but are also massed on the spawning grounds and are particularly vulnerable to capture. The most controversial regulation has been the catch quota which was formally applied in 1936-7 to most of the major fishing areas of the province as a safeguard against "excessive" fishing which might lead to a progressive decline in the stocks.

The need for catch quotas has frequently been questioned, particularly by fishermen who are familiar with the European herring fisheries which have been exploited for centuries without major catch restrictions, and particularly also by the operators of herring canneries and reduction plants, who in good seasons naturally are loath to stop fishing operations while there is still an apparent abundance of fish. Thus the main issues confronting the scientific investigator are these: are present catch restrictions necessary; if necessary, are they adequate; if adequate, are they the best that can be devised to produce maximum sustained catch?

It is the purpose of this article to review the history of the west coast of Vancouver Island fishery where certain earlier indications of a decline in the supply led to the general application of the quota system of regulation, to interpret recent fluctuations in catch in the light of presently available biological information, and to describe and give preliminary results of an investigation which has been designed to test the efficacy of catch limitations.

STATISTICAL AREAS

The west coast of Vancouver Island sub-district has been divided into five areas (Fig. 1) which for brevity will be referred to henceforth by number:

Area 23:	Pachena Point to Quisitis Point, including Barkley Sound and contiguous waters.
Area 24:	Quisitis Point to Estevan Point, including Clayoquot Sound, Sydney Inlet, Hesquiat Harbour, etc.
Area 25:	Estevan Point to Tatchu Point, including Nootka Sound, Tahsis Inlet, Esperanza Inlet, Nuchatlitz Inlet, etc.
Area 26:	Tatchu Point to Cape Cook, including Kyuquot Sound, Nasparte, Ououkinsh, and Malksope Inlets, etc.
Area 27:	Cape Cook to Cape Scott, including Quatsino Sound, Klaskish and Klaskino Inlets, etc.

THE HERRING FISHERY

Herring fishery on the west coast of Vancouver Island usually starts towards the end of October and lasts until February 5, with a "lay-off" of about ten days over the Christmas—New Year holiday season. However, the exact length of the fishing season depends on several factors such as the success of fishing, the reaching of catch quotas, or the closure of the season earlier or later than February 5 by government regulation.

The fishery operates in inshore waters on only a part of the total herring population, namely, schools of maturing and mature fish which start to congregate within the sounds and inlets several months prior to the main spawning period (middle of February to the end of March).

The fish are caught by a fleet of "seine boats" using "purse seines." Modern seine boats range from about 65 to 80 feet in length and from

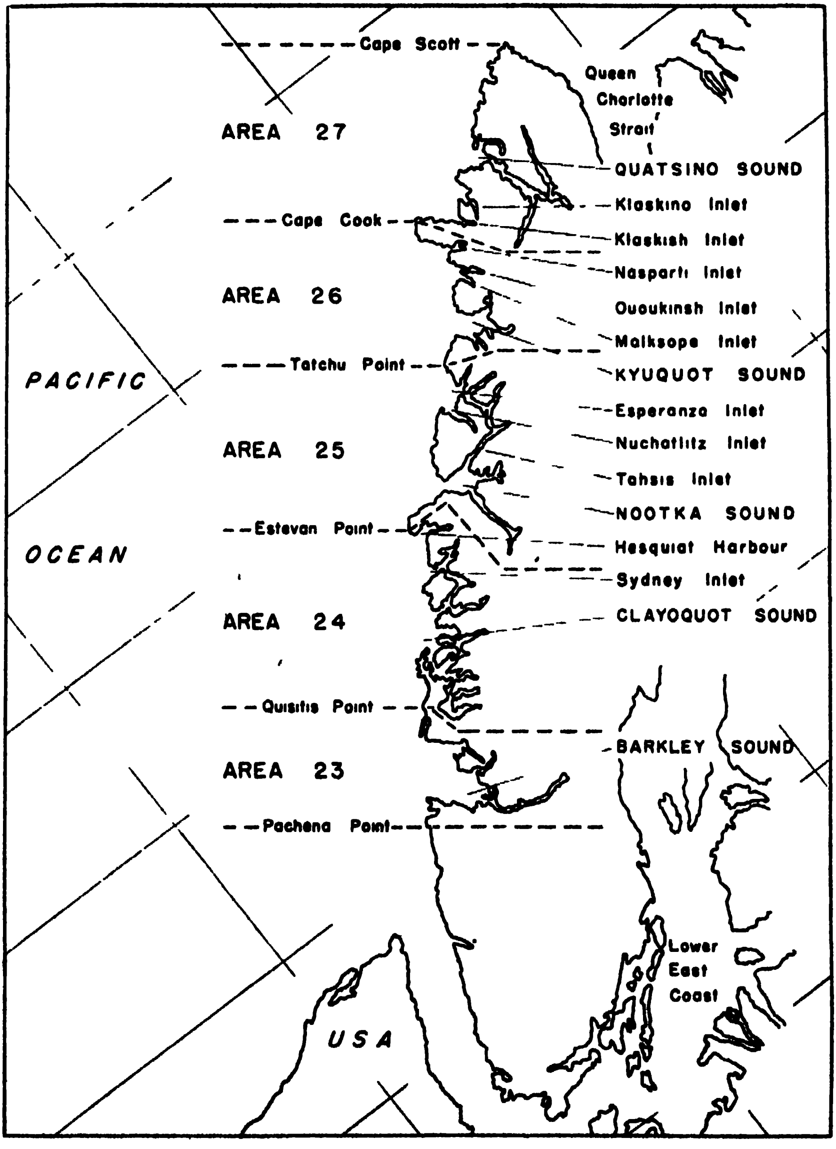


FIGURE 1.—Map of the west coast of Vancouver Island showing the boundaries of areas and the location of places mentioned in the text.

about 60 to 100 (gross) tons in weight. The purse seines, with a web of 1½-inch (stretched) mesh, are up to 275 fathoms in length, and fish effectively to a maximum depth ranging from 20 to 35 fathoms. The fish are located by "feeling wire" or, more recently, by echo sounder (Tester, 1943). Once the fish are located, the seine boat encircles them, allowing the seine to run off the stern "table" on which it is piled; the seine is drawn together at the bottom, or "pursed"; the web is gradually hauled aboard, or "fleeted"; and the fish are gradually confined to one end of the seine which is made of small-meshed heavy web, called the "bunt." The fish are then dipped, or "brailed" from the bunt and loaded on "packers" which carry the catch to the canneries or reduction plants. An average "set" of the seine would yield about 50 tons of fish, although sets yielding up to 1,200 tons have been made.

There has been a considerable increase in the efficiency of fishing within recent years. The fishermen have gained in experience, larger seine boats have come into use, the seines have increased in length and depth, packer and plant capacities have increased, scouting time has been reduced, and fishing time has been increased by the introduction of the radio telephone for communication and the echo sounder for locating the fish (Tester, 1943). The latter instrument, first used on a large scale about 1945-6, has also opened up new grounds within the areas both by showing the presence of fish and by disclosing new spots on uneven bottom where sets could be made without danger of snagging the seine.

Catch Statistics and History

The history of the west coast of Vancouver Island herring fishery has been discussed in detail elsewhere (Tester, 1935 and 1945b), but is summarized here as a background for the present study. The total catch for the sub-district and the catch for Area 23 alone from 1910-11 to 1945-6 are both shown in Fig. 2 (Table 1).

From 1910-11 to 1919-20 (Period A) when the fishery was in its infancy the catch was relatively small, averaging less than 3,000 tons annually prior to 1916-17, but undergoing a considerable increase following that year with the development of a large-scale fishery to satisfy a temporary wartime demand for food products and more permanent Oriental markets for dry salt herring.

From 1920-1 to 1927-8 (Period B) the catch was large, averaging about 30,000 tons annually, mostly taken from Area 23. A high fishing effort was maintained to supply fish for a flourishing dry salt industry. Towards the end of the period exploratory fishing took places in Areas 24 to 26 with some success, the catch mostly being reduced to oil and meal in newly-built pilchard reduction plants. Except in these newly-fished areas, fishing was not hampered by catch restrictions; usually sufficient fish to satisfy dry salt requirements were taken in Area 23

TABLE I

CATCH OF HERRING IN THOUSANDS OF TONS FROM 1910-11 TO 1945-6

<table>
<tr><th></th><th colspan="5">Area</th><th></th></tr>
<tr><th><i>Year</i></th><th>23</th><th>24</th><th>25</th><th>26</th><th>27</th><th><i>Total</i></th></tr>
<tr><td>1910-11</td><td>+</td><td colspan="2">+</td><td colspan="2">−</td><td>+</td></tr>
<tr><td>1911-12</td><td>0.4</td><td colspan="2">0.5</td><td colspan="2">−</td><td>0.9</td></tr>
<tr><td>1912-13</td><td>0.9</td><td colspan="2">0.5</td><td colspan="2">0.5</td><td>1.9</td></tr>
<tr><td>1913-14</td><td>2.1</td><td colspan="2">+</td><td colspan="2">+</td><td>2.1</td></tr>
<tr><td>1914-15</td><td>3.7</td><td colspan="2">+</td><td colspan="2">+</td><td>3.7</td></tr>
<tr><td>1915-16</td><td>2.6</td><td colspan="2">+</td><td colspan="2">+</td><td>2.6</td></tr>
<tr><td>1916-17</td><td>2.5</td><td colspan="2">+</td><td colspan="2">+</td><td>2.5</td></tr>
<tr><td>1917*</td><td>3.6</td><td>+</td><td>−</td><td>−</td><td>−</td><td>3.6</td></tr>
<tr><td>1918*</td><td>11.7</td><td>−</td><td>−</td><td>+</td><td>−</td><td>11.7</td></tr>
<tr><td>1919*</td><td>20.0</td><td>−</td><td>−</td><td>−</td><td>−</td><td>20.0</td></tr>
<tr><td>1920*</td><td>32.0</td><td>−</td><td>−</td><td>−</td><td>−</td><td>32.0</td></tr>
<tr><td>1921*</td><td>23.5</td><td>−</td><td>−</td><td>−</td><td>−</td><td>23.5</td></tr>
<tr><td>1922*</td><td>21.5</td><td>−</td><td>−</td><td>−</td><td>−</td><td>21.5</td></tr>
<tr><td>1923*</td><td>32.1</td><td>−</td><td>0.2</td><td>−</td><td>−</td><td>32.3</td></tr>
<tr><td>1924*</td><td>35.4</td><td>−</td><td>0.9</td><td>−</td><td>−</td><td>36.3</td></tr>
<tr><td>1925*</td><td>31.1</td><td>−</td><td>−</td><td>−</td><td>−</td><td>31.1</td></tr>
<tr><td>1926-7</td><td>21.8′</td><td>−</td><td>−</td><td>1.9</td><td>−</td><td>23.7</td></tr>
<tr><td>1927-8</td><td>29.8′</td><td>−</td><td>−</td><td>14.0</td><td>−</td><td>43.8</td></tr>
<tr><td>1928-9</td><td>12.0′</td><td>−</td><td>0.4</td><td>6.7</td><td>+</td><td>19.1</td></tr>
<tr><td>1929-30</td><td>7.5′</td><td>1.0</td><td>1.0</td><td>2.8</td><td>0.3</td><td>12.6</td></tr>
<tr><td>1930-1</td><td>13.0′</td><td>0.5</td><td>10.6</td><td>3.9</td><td>0.2</td><td>28.2</td></tr>
<tr><td>1931-2</td><td>6.4′</td><td>17.8</td><td>0.5</td><td>5.0</td><td>+</td><td>29.7</td></tr>
<tr><td>1932-3</td><td>5.5′</td><td>6.6</td><td>1.0</td><td>6.4</td><td>+</td><td>19.5</td></tr>
<tr><td>1933-4</td><td>20.0</td><td>0.6</td><td>+</td><td>1.1</td><td>1.2</td><td>22.9</td></tr>
<tr><td>1934-5</td><td>7.0</td><td>−</td><td>−</td><td>−</td><td>0.2</td><td>7.2</td></tr>
<tr><td>1935-6</td><td>13.3</td><td>−</td><td>3.1</td><td>8.4</td><td>−</td><td>24.8</td></tr>
<tr><td>1936-7</td><td>20.8</td><td>3.8</td><td>5.3</td><td>10.0</td><td>1.7</td><td>41.6</td></tr>
<tr><td>1937-8</td><td>15.1</td><td>0.2</td><td>3.8</td><td>6.1</td><td>5.1</td><td>30.3</td></tr>
<tr><td>1938-9</td><td>6.3</td><td>0.7</td><td>2.2</td><td>0.8</td><td>5.1</td><td>15.1</td></tr>
<tr><td>1939-40</td><td>6.9</td><td>+</td><td>3.9</td><td>4.2</td><td>1.5</td><td>16.5</td></tr>
<tr><td>1940-1</td><td>13.4</td><td>0.9</td><td>5.8</td><td>4.9</td><td>0.1</td><td>25.1</td></tr>
<tr><td>1941-2</td><td>2.3</td><td>1.1</td><td>5.0</td><td>5.7</td><td>3.0</td><td>17.1</td></tr>
<tr><td>1942-3</td><td>0.9</td><td>2.6</td><td>3.6</td><td>6.2</td><td>3.5</td><td>16.8</td></tr>
<tr><td>1943-4</td><td>4.2</td><td>4.9</td><td>−</td><td>+</td><td>+</td><td>9.1</td></tr>
<tr><td>1944-5</td><td>5.9</td><td>6.1</td><td>8.1</td><td>0.2</td><td>0.1</td><td>20.4</td></tr>
<tr><td>1945-6</td><td>21.3</td><td>4.6</td><td>1.1</td><td>0.3</td><td>0.2</td><td>27.5</td></tr>
</table>

*Catch for calendar year which, in this case, coincides approximately with the fishing season.

′Approximate.

well before the prescribed closing date of February 5. There were fluctuations in catch but apparently no pronounced scarcities of fish.

From 1928-9 to 1934-5 (Period C) the catch fluctuated about a lower level than previously, averaging about 20,000 tons and reaching the low figure of 7,200 tons in 1934-5. The decline was due partly to decreased fishing effort in Area 23 as a result of the loss of markets for the dry salt product (Tester, 1945b, p. 38). However, even with decreased fishing effort, the supply of fish in this area seemed to be more erratic than in former years. The fishery continued to expand in Areas 24 to 27 but in some years fishing effort was curtailed by time limitations, which were imposed "for purposes of conservation," but which were motivated both by fear of overfishing and prejudice against reduction. Reduction licences were not issued in 1934-5 and no fishing took place in Areas 24 to 27; hence the low total catch in that year.

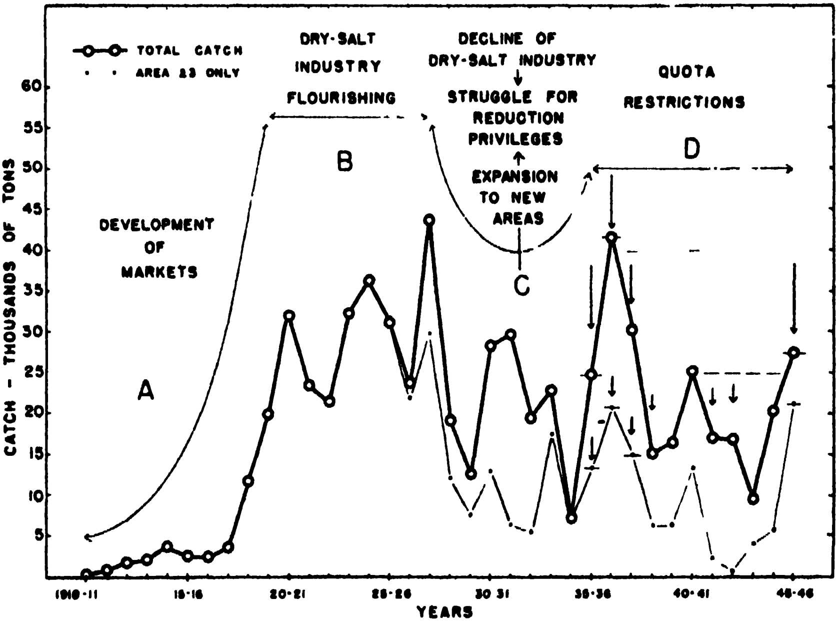


FIGURE 2.—Graph showing fluctuations in catch over the period 1910-11 to 1945-6, with an indication of some of the factors affecting fishing effort.

Allowing for curtailment of fishing effort during Period C, it seems evident that there were scarcities of fish in some areas and years and that the newly exploited areas failed to produce the large catches which might have been expected from new grounds. As the fishery expanded, catches of 10,000 tons or more were made but once in each new area, and in each case the year of initially good fishing was followed by years of fair and poor fishing. In no one area was the catch as consistently large as in the early years of fishing in Area 23.

Fom 1935-6 to 1945-6 (Period D), the catch averaged about 22,000 tons. Fear of depletion, together with the desirability of stabilizing regulations, stimulated the imposition of a catch-quota system of restriction and this, rather than the method of disposal of the catch, became the limiting factor to fishing effort (Tester, 1945b, pp. 9-10). In 1935-6 an unofficial quota of 25,000 tons, half to be taken from Area 23, was established. From 1936-7 to 1940-1 a "blanket" quota of 40,000 tons was imposed, with sub-quotas for Areas 23 to 27 as follows: 15,000, 5,000, 10,000, 10,000, and 5,000 tons. From 1941-2 to 1945-6 the blanket quota was reduced to 25,000 tons and sub-quotas were eliminated except for Area 27, which was maintained but reduced to 3,000 tons.

Apart from an extension of 5,000 tons for Area 23 in 1936-7, the above quotas and sub-quotas were maintained over Period D. In addition, however, the closing date of February 5 was retained. The Chief Supervisor of Fisheries also had the authority to close fishing at any time for purposes of conservation and this power was exercised in certain years of apparent low abundance to enforce early closures, in other years, time extensions beyond February 5 were granted (Tester, 1945b, Table VII, p. 42).

In Fig. 2 quota restrictions are indicated by bars, and curtailment of fishing effort when quotas or sub-quotas were reached, by arrows. For the west coast as a whole, the catch reached a peak of over 40,000 tons in 1936-7, the first year of official quota regulation. In subsequent years it declined to about 15,000 tons in 1938-9, increased to about 25,000 tons in 1940-1, again decreased to about 9,000 tons in 1943-4, and again increased to about 27,000 tons in 1945-6. The blanket quota (40,000 tons) was reached at the beginning of the period (1936-7) and the reduced blanket quota (25,000 tons) was reached at the end of the period (1945-6). Sub-quotas were reached in 1936-7 (Areas 23 and 26), 1937-8 (Areas 23 and 27), 1938-9 (Area 27), 1941-2 (Area 27), and 1942-3 (Area 27), but not in other years and areas.

Consideration of the graph for the period from 1920-1 to 1945-6 would not necessarily indicate that a general decline in population abundance for the west coast as a whole has taken place, particularly when restrictions to fishing effort in Periods C and D are taken into consideration. However, the graph does indicate that fluctuations in population abundance have been more violent in the recent Period D than in the early Period B. It might be argued that a general progressive decline has taken place but is not apparent from total catch figures because of the expansion of the fishery to include new populations. In support of this argument the graph for Area 23 might be cited as proof of a local decline in abundance, perhaps stressing the consistently poor fishing from 1938-9 to 1944-5 as compared with the consistently good fishing from 1920-1 to 1928-9. This possibility will be discussed later.

Superficial examination of the graph suggests a periodicity in the catch fluctuations, with a minor cycle of about four or five years superimposed on a major cycle of about twice that frequency. Although the catch has been subject to varying restrictions which complicate the interpretation of the graph, the possibility of cyclic fluctuations in abundance cannot be denied.

EXTENT OF MIXTURE BETWEEN RUNS

Early investigations of "racial" characters, such as the average number of vertebrae in the backbone of herring caught on various fishing grounds, indicated the presence of local populations of herring along the west coast of Vancouver Island (Tester, 1936 and 1937a). A general gradation in mean vertebral number, together with certain mean differences which were greater for areas separated from each other and less for closely-situated or adjacent areas, suggested that mixing was limited or absent between at least some of the west coast runs. However, it was realized that from this type of data it is difficult to designate boundaries for local populations and impossible to determine the amount of "limited" mixing which might take place between them.

When quotas were established in 1936-7, sub-quotas were set for each of the five statistical areas on the assumption that each supported an essentially independent run. It was realized that this assumption might not be strictly true and it was to be made the subject of further examination by newly developed methods of study. These involved the use of internal metal tags and their recovery by means of electromagnets in the meal lines of reduction plants and electronic tag detectors in the unloading systems of both reduction plants and canneries.

Tagging of west coast of Vancouver Island herring took place each year from 1936-7 to 1940-1, and again to a limited extent in 1943-4. The results for each year have been presented and discussed in detail (Hart and Tester, 1937, 1938, 1939, and 1940; Hart, Tester, and McHugh, 1941; Hart, Tester, and Boughton, 1942; Tester and Boughton, 1943; Tester, 1944, 1945, and 1946) and have recently been combined and summarized (Tester, MS). The main conclusions will be reviewed briefly here.

The west coast of Vancouver Island herring may be considered to form a major population which is essentially distinct from other major populations which support the fisheries along the lower east coast of Vancouver Island, Queen Charlotte Strait, the Central coastline, etc. The amount of mixture is small, on the average probably involving less than 5 per cent of the population.

The runs to the five areas along the west coast of the island constitute a series of intergrading populations, each of which exhibits a definite "homing" tendency but each having a relatively small degree of independence. The extent of mixture is greatest between runs to adjacent areas, and is progressively less the more widely separated are the areas. The average dispersal or "wandering" from each area probably involves about 45 per cent of the fish which spawned there and the extent of dispersal varies with both year and area.

In Fig. 3 an attempt is made to illustrate average conditions pictorially, showing fish of the various runs scattered on offshore feeding grounds during the summer, with the extent of mixture roughly proportional to that indicated by tag returns and with arrows showing their inshore movement during the fall and winter and their offshore movement after spawning in the spring. The location and distribution of the fish on the summer feeding grounds is hypothetical but is supported by circumstantial evidence such as the presence of herring in the stomachs of fur seals taken offshore (Clemens, Hart, and Wilby, 1936).

The conclusions are based on tags used during the spring spawning season and recovered during subsequent winter fishing seasons. There is no reliable information on movements which might take place between areas during the fishing season. Observations would indicate that in general once a run enters an inlet it remains there until spawning time, although there have been cases of the "disappearance" of a large body of fish during the fishing season.

It should be stressed that the conclusions are based on the tagging of mature and maturing fish of the population. It is possible that more extensive mixing may take place during larval or juvenile stages, although vertebral data would indicate that this is not the case (Tester, MS).

With regard to the history of the west coast of Vancouver Island herring fishery it seems certain that in the early period of intensive fishing (Fig. 2*B*), when activities were almost entirely confined to Area 23, both the population and the catch in that area were bolstered by substantial additions of fish which spawned in previous years in the unfished areas to the northwest, particularly Area 24, and to a lesser extent Areas 25, 26, and 27 in order of importance. When these areas themselves were later subject to exploitation this source of supply to

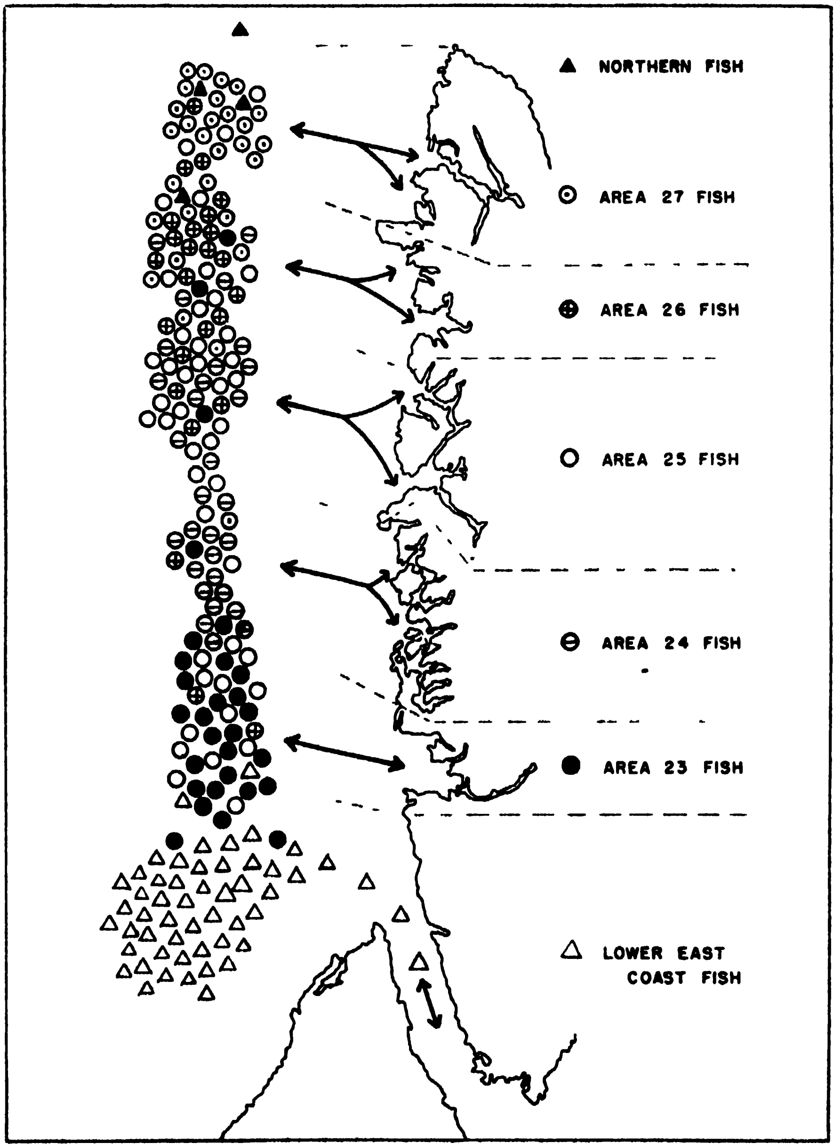


FIGURE 3. - Diagram to illustrate the series of intergrading runs along the west coast of Vancouver Island, with the extent of mixture as indicated by tag returns. The runs are shown on offshore feeding grounds where they are presumed to pass the summer.

Area 23 would be reduced, thus contributing to the relatively more erratic fishing in recent as compared with early years.

As the degree of independence of individual runs is relatively weak, for practical purposes they will be considered as belonging to one major population which will henceforth be treated as a unit.

Fluctuations in Availability in Recent Years

Detailed catch statistics, consisting of daily records of the fishing activity of individual seiners, enable fluctuations in the "availability" of herring to the fishermen to be traced. Such records have been collected over the period 1933-4 to 1945-6 and have been presented and discussed in part elsewhere (Tester, 1945b).

In Table II is given the following information for each year: approximate number of seines, number of active fishing days expended by all boats (adjusted to compensate for missing records), total catch (from Table I), and the average catch per seine per day, or availability. The data are illustrated in Fig. 4.

TABLE II

Fishing Effort, Catch, and Availability

Year	*No. of seines (approximate and minimal)*	*Effort (no. of active fishing days)*	*Catch (thousands of tons)*	*Availability (av. catch/seine/day)*
1933-4	19	519	22.9	44.1
1934-5	5	119	7.2	60.4
1935-6	13	365	24.8	67.9
1936-7	19	818	41.6	50.9
1937-8	18	858	30.3	35.3
1938-9	16	848	15.1	17.8
1939-40	20	907	16.5	18.2
1940-1	20	1,028	25.1	24.4
1941-2	13	814	17.1	21.0
1942-3	15	620	16.8	27.1'
1943-4	14	550	9.1	16.5'
1944-5	16	592	20.4	34.5
1945-6	24	576	27.5	47.7

'Adjusted; compare Tester, 1945b, Table V, p. 23.

As seine boats have spent varying lengths of time in the sub-district, the number of active fishing days is considered to be a better measure of fishing effort than the number of seines. It will be observed (Fig. 4*A*) that fishing effort was relatively low in 1934-5 and 1935-6, that it was high over the period 1936-7 to 1941-2, and apparently fell to a lower level over the period 1942-3 to 1945-6. The recent apparent decrease in fishing effort is associated with a tendency for the runs to appear later in the season, with the result that the main portion of the fleet either delayed its arrival or fished "homestead" pilchards while waiting for the herring. However, if the gradually increasing efficiency of effort is taken into consideration, particularly that resulting from the introduction and use of echo sounders for scouting in 1944-5 and 1945-6, the true effort may be considered to have been approximately constant from 1936-7 to 1945-6.

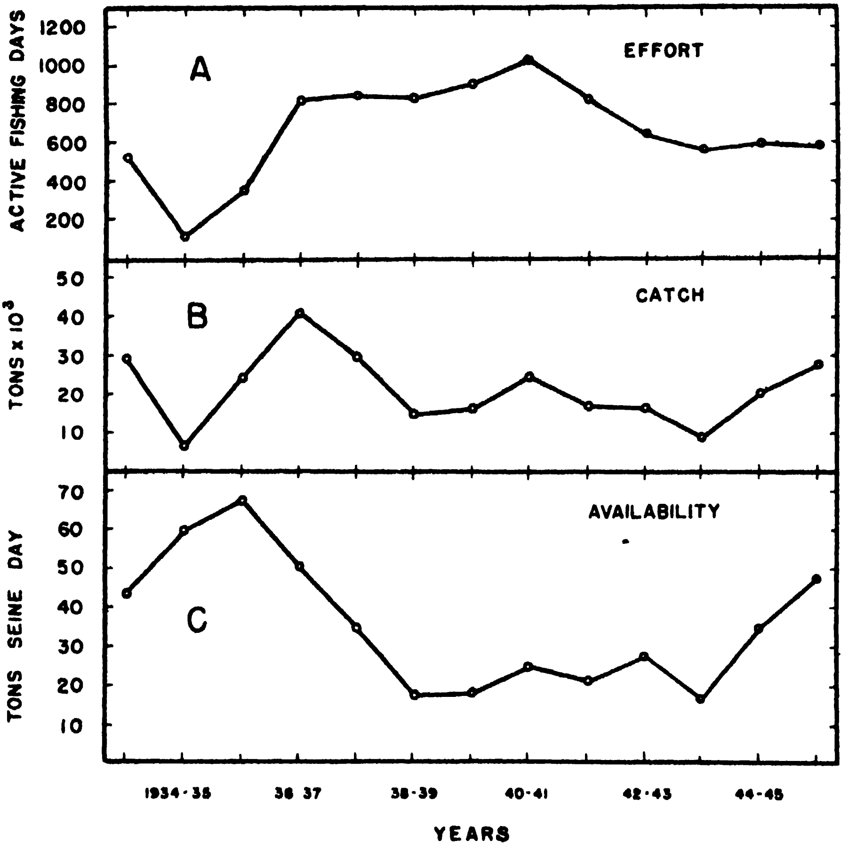


FIGURE 4.—Effort (number of active fishing days), catch (in thousands of tons), and availability (average catch per seine per day's active fishing) over the period 1933-4 to 1945-6.

If true effort may be regarded as approximately constant, total catch (Fig. 4*B*) may be considered to represent fluctuations in the abundance of that portion of the fishable stock which has entered inshore waters each year from 1936-7 to 1945-6. However, in order to make use of data over the whole period 1933-4 to 1945-6, catch per unit effort, or availability (Fig. 4*C*) is taken as the index of abundance realizing, however, that it may be biased upwards to some extent by the gradually increasing efficiency of effort. It will be noted that abundance was high in both 1934-5 and 1935-6 when fishing effort was rather drastically curtailed by regulation. It then progressively decreased to a low value in 1938-9 and remained low, with minor fluctuations, until 1943-4. It again increased during 1944-5 and 1945-6, in which later year effort was again curtailed with the reaching of the catch quota.

FLUCTUATIONS IN SPAWNING IN RECENT YEARS

Some idea of fluctuations in the abundance of that portion of the fishable population which escapes the fishery, including the schools which enter inshore waters after the close of the fishing season, may be obtained from observations on spawning.

On the west coast of Vancouver Island the main spawning period occurs after the close of the fishing season and extends from about the middle of February to the end of March. However, early spawnings in January and late spawnings in April occasionally take place. The eggs are usually deposited on vegetation such as eelgrass, rockweed, and kelp, which grows within or just below the intertidal zone. Thus a large part of the spawning grounds is exposed and can be examined on a low spring tide.

For several years inspectors of the Dominion Department of Fisheries have submitted annual reports on herring spawning grounds. During earlier years (1931 to 1936), as time permitted, they made observations on as many individual spawnings as came to their attention, and submitted reports on these together with their estimate of the "success" (intensity) of spawning in the areas which they patrolled. Thus for a given area spawning might be classed as very light, light, medium, heavy, or very heavy. In recent years (1937 to 1946) efforts were made to obtain more detailed, complete, accurate, and uniform information on herring spawning. Spawning ground surveys were recognized as an important phase of the inspector's work and more time and facilities were placed at his disposal. His duties consisted of patrolling an area during the entire spawning period, examining spawnings as closely as possible, and submitting information on the date, length, average width, depth, and intensity of egg deposition for each individual spawning, together with observations on loss of eggs from various sources, weather conditions during the spawning and incubation period, etc. He was provided with printed forms on which to record the observations.

Despite the added time and care which has been given to herring spawning surveys in recent years, through no fault of the observers there remain many sources of error: small spawnings may be missed; length estimates may be faulty because of the "patchy" distribution of the vegetation on which the eggs are deposited; average width estimates are very rough because of the difficulty of sampling adequately below the low tide level; intensity estimates are subject to personal bias, and so on. However, it is believed that the data, as presented, are sufficiently accurate to trace major annual fluctuations in egg deposition and in the quantity of spawning fish.

For present purposes use is made of the fisheries inspectors' estimates of the general intensity of spawning in each area over the period 1931 to 1946, summing those for the various areas in each year to obtain an index for the sub-district as a whole (Table III). This, Index A, follows fairly closely ($r = 0.76$; $P = 0.01$) a more elaborate one, Index B, which was calculated by multiplying the miles of spawn by the estimated intensity (categories numbered 1 to 5) for individual spawnings and summing these for each year over the period 1937 to

TABLE III

SPAWNING INDICES FROM 1931 TO 1946
(for explanation see text)

	Intensity in Area					*All Areas*	
Year	23	24	25	26	27	*Index A*	*Index B*
1931	2	5	4	2	4	17	–
1932	2	4	1	1	2	10	–
1933	4	2	2	1	3	12	–
1934	5	3	3	2½	3½	17	–
1935	1	3½	4	4	4	16½	–
1936	2	1	5	5	5	18	–
1937	3½	1	2	4	2	12½	104.0
1938	1	2	1	3	2	9	31.6
1939	4	2	2	3	2	13	69.7
1940	2	3	3	2	2	12	78.5
1941	3	2	4	1	2½	12½	72.1
1942	2	2	2½	3	3	12½	33.5
1943	1	4	1½	1	1	8½	39.3
1944	2	4	3½	2	2	13½	36.0
1945	4	4	3½	3	3	17½	159.7
1946	4	5	4	1	3	17	103.4

1946, and is perhaps just as accurate; it has the advantage that data over a longer period can be considered.

Fluctuations in the spawning indices are shown graphically in Fig. 5*A*. According to Index A, spawning was relatively good in 1931, 1934, 1935, 1936, 1945, and 1946, whereas it was relatively poor in 1932, 1938, and 1943. Index B suggests that it was also poor in 1942 and 1944. In other years it might be classed as fair or average. Comparison of values for Index A from 1934 to 1946 (Table III) with those for availability from 1933-4 to 1945-6 (Table II) shows that fluctuations in the spawning population roughly follow those in that portion of the population contributing to the fishery ($r = 0.58$; $P = 0.05$). Certainly in years of low catch and availability there was no tremendous increase in the spawning population, showing that scarcities during the fishing season were not mainly due to failure of the fishable stock to enter inshore waters during the fishing season.

The spawning index shows fluctuations from year to year but no pronounced downward trend. If a downward trend has occurred, it is doubtful if it would be shown by the data. There would likely be a gradually changing standard of intensity on the part of the observers; for example, a spawning classed as "medium" in early years might be classed as "heavy" in later years.

Figs. 5*B* and 5*C* will be discussed later.

Growth in Weight

Information on the increase in weight of herring as they grow older is pertinent to this discussion. In Table IV is included the average

TABLE IV

Average Weight and Weight Increment (grams) according to Age for Herring of Area 23 over a Sixteen Year Period

Age	*Number of fish*	*Average weight*	*Smoothed average weight*	*Weight increment*	*Percentage weight increment*
I	25	15.88	16		
II	605	52.22*	52	36	225.0
III	4,038	87.03*	87	35	67.3
IV	2,161	109.03*	109	22	25.3
V	873	124.99*	125	16	14.7
VI	284	138.73	138	13	10.4
VII	62	150.42	148	10	7.2
VIII	7	150.43	156	8	5.4
IX	4	167.75	163	7	4.5

*Unweighted average of means.

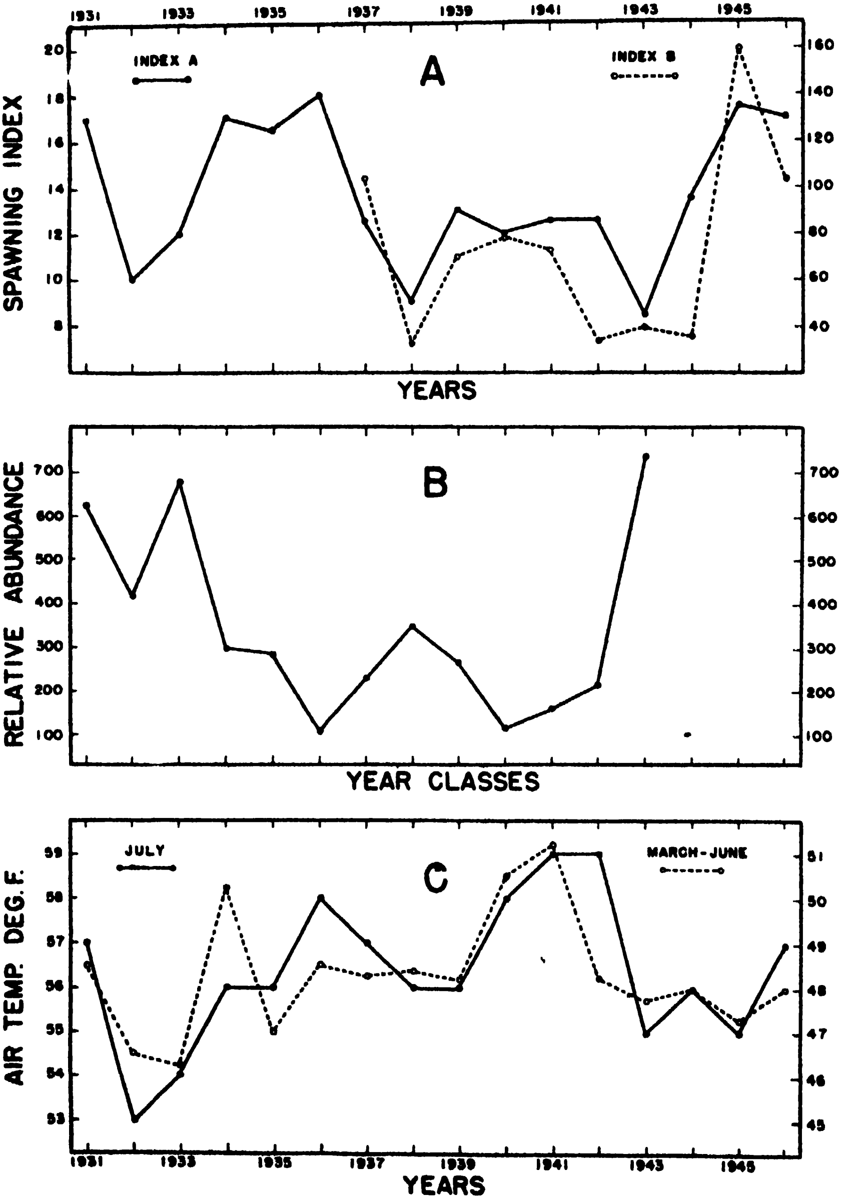


FIGURE 5.—Graphs showing fluctuations in the following: *A*—spawning intensity, Index A over the period 1931 to 1946 and Index B over the period 1937 to 1946; *B*—relative abundance of year classes 1931 to 1943; and *C*—average air temperatures for the combined months of March to June and for July at Estevan Point.

weight according to age over a sixteen year period (1929-30 to 1944-5) for fish caught in Area 23. For present purposes the data, smoothed by eye in Fig. 6, may be considered representative for all west coast of Vancouver Island areas. Ages were determined from the scales of samples of herring from the catches (Tester, 1937b, p. 148).

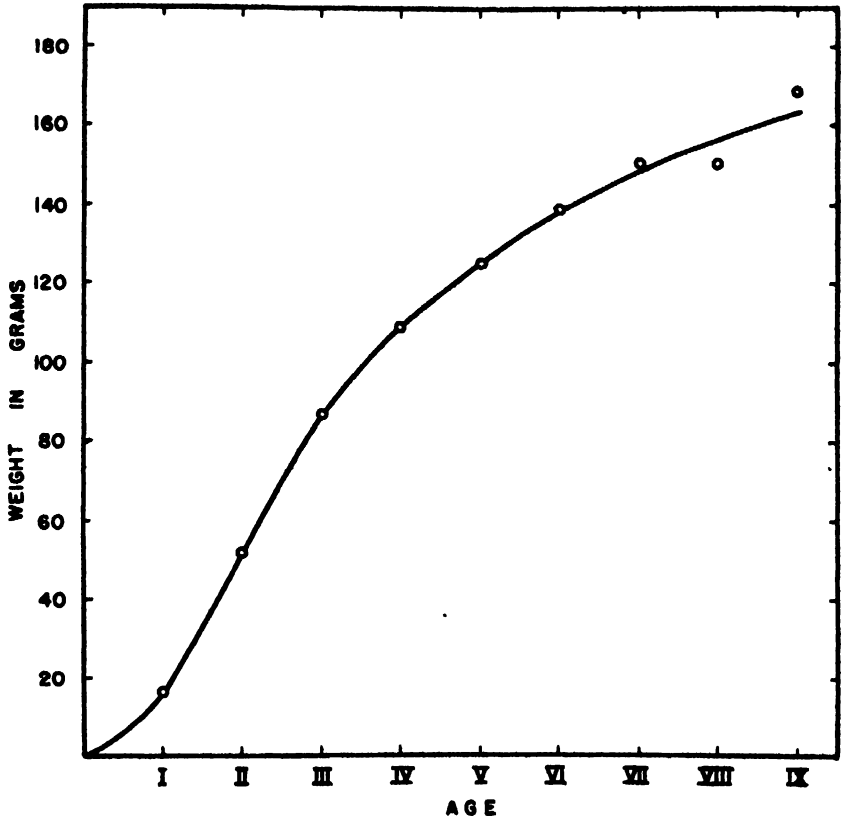


FIGURE 6.—Curve showing increase in average weight of herring with increase in age.

Growth is much more rapid in early than in later years of life. The weight increment is large between ages I and II (225 per cent), but decreases rapidly and consistently with increasing age to a very small increment (4.5 per cent) between ages VIII and IX. Growth in the herring is in marked contrast to that in the Pacific halibut (Thompson and Bell, 1934, p. 30) where it was found that increase in weight took place at a fairly constant rate of about 21 per cent per annum between ages IV and XI.

An increase in growth rate appears to have taken place in recent years. This is shown by data in Table V giving the average weight at each age over an early eight year period (1929-30 to 1936-7) and a later eight year period (1937-8 to 1944-5). The change in rate of growth in weight has been accompanied by a corresponding change in rate of growth in length, the fish being larger as well as heavier for a given age during the recent period. It seems unlikely that this difference is due to lack of uniformity in the technique of age determination. One possible explanation is that growth has been accelerated at lower population abundance during the recent period. The biological implication is that competition for food may be a limiting factor for growth in the sea, a suggestion which was earlier made by Petersen (1922) who observed a similar increase in the growth rate of plaice of the western Baltic Sea with decreased population abundance.

TABLE V

AVERAGE WEIGHT (GRAMS) ACCORDING TO AGE FOR HERRING OF AREA 23 DURING TWO EIGHT YEAR PERIODS

	1929-30 to 1936-7		1937-8 to 1944-5	
Age	*Number of fish*	*Weight*	*Number of fish*	*Weight*
II	95	45.3	512	62.8
III	2,549	83 0	1,489	94.0
IV	1,255	105.2	905	114.1
V	440	120.6	433	124.4
VI	166	137.6	117	140.4

FLUCTUATIONS IN THE ABUNDANCE OF SUCCESSIVE YEAR CLASSES

Sampling of the herring catches along the west coast of Vancouver Island has been underway since 1929-30 and it has been found that the age composition of the samples varies considerably from area to area in the same year (Tester, 1937b, p. 156). In order to present a set of data for the sub-district as a whole, the percentage age composition for each area has been weighted to the catch in each area and then averaged for all areas each year. Aside from minor irregularities and gaps in the data, the resultant percentage age composition, given in Table VI, may be considered for present purposes representative of the catch in each year for the sub-district as a whole.

It is obvious from the table that successive year classes have varied considerably in relative abundance. For example, fish of the 1931 year class formed 60.01 per cent of the catch at age III in 1933-4, whereas fish of the 1930 year class formed but 38.13 per cent of the catch at the same age in 1932-3; the relatively greater abundance of the 1931 over the 1930 year class persisted in succeeding years (age IV—42.31 compared with 20.31 per cent, age V—13.09 compared with 5.84 per cent, etc.).

In order to obtain figures roughly proportional to the actual abundance of each year class at each age, the availability values given in Table II were converted from weight to numbers of fish, using the data of Tables IV and VI, and the numbers thus obtained were allocated to the various age groups. For example, for 1933-4 it was calculated that the average weight of a fish was 98.6034 grams, and that in 44.1 tons there were 405,646 (406 × 10^3) individuals which, according to the data of Table VI, were distributed as follows: 9,939 (10 × 10^3) at age II, 243,453 (243 × 10^3) at age III, etc. In order to make full use of the available data the contributions of year classes at ages not yet sampled were estimated, assuming that the decrease in numbers from one age to the next would be in proportion to the average

TABLE VI

PERCENTAGE AGE COMPOSITION, WEIGHTED TO CATCH IN EACH AREA, OVER THE PERIOD 1929-30 TO 1945-6

					Age						*Number*
Year		II	III	IV	V	VI	VII	VIII	IX	X	*of fish*
1929-30	0.12	1.37	71.39	19.90	4.98	1.99	0.12	0.12	–	–	804
1930-1	–	2.73	54.89	38.11	4.07	0.23	–	–	–	–	378
1931-2	–	0.50	33.38	36.91	27.98	1.09	–	–	–	–	2,184
1932-3	–	1.11	38.13	29.78	19.81	10.83	0.24	–	0.01	–	2,016
1933-4	–	2.45	60.01	20.31	8.06	6.49	2.67	–	–	–	1,239
1934-5	–	0.20	46.29	42.31	5.84	2.39	1.06	0.13	–	–	754
1935-6	–	2.65	66.02	16.71	13.09	1 25	0.14	0.14	–	–	718
1936-7	0.05	4.31	15.75	38.26	7.03	3.87	0.34	0.17	0.17	0.03	2,523
1937-8	–	4.34	56.57	17.07	16.19	3.27	1.79	0.35	0.22	0.14	2,372
1938-9	–	31.47	21.96	29.26	5.47	6.17	1.12	1.03	0.33	0.05	2,008
1939-40	0.02	34.28	42.16	12.46	8.78	1.24	0.72	0.14	0.20	–	3,665
1940-1	–	3.54	58.97	25.80	8.52	2.59	0.37	0.17	0.04	–	3,857
1941-2	–	2.72	55.75	32.06	7.04	1.87	0.48	0.07	0.02	–	1,437
1942-3	–	4.38	22.63	39.12	25.84	6.03	1.52	0.42	–	–	2,200
1943-4	–	11.24	43.91	17.07	18.71	7.56	1.34	0.15	0.03	–	2,099
1944-5	0.75	26.99	35.26	17.89	7.99	8.08	2.78	0.15	0.09	–	3,160
1945-6	–	10.84	73.32	10.12	3.21	1.26	1.06	0.17	0.02	–	4,635

decrease for the same ages in the older year classes. For example, the 1943 year class contributed 360,196 (360×10^3) fish at age III in 1945-6. Its contribution at age IV in 1946-7 was estimated at 360,196 (the number at age III) multiplied by 1,000,504 (the total number contributed at age IV by year classes 1931 to 1942) divided by 1,934,849 (the total number contributed at age III by the same year classes), or 186,256 (186×10^3). The figures thus obtained are shown in Table VII, together with the sums of the contributions of each year class from

TABLE VII

RELATIVE ABUNDANCE OF EACH YEAR CLASS AT EACH AGE
(for explanation see text)

Year class	Age									*Total*
	I	II	III	IV	V	VI	VII	VIII	IX	
1927	?	?	?	?	?	?	11	1	–	?
1928	?	?	?	?	?	26	6	1	1	?
1929	?	?	?	?	33	13	1	1	1	?
1930	?	?	?	82	32	8	2	1	1	?
1931	?	39*	243	232	84	18	6	2	+	621
1932	?	10	254	108	33	11	2	+	+	418
1933	–	11	426	178	53	11	1	+	+	680
1934	–	17	213	55	10	2	1	+	–	298
1935	–	20	184	53	18	6	1	1	+	283
1936	+	14	45	25	19	4	3	+	+	110
1937	–	57	84	59	14	13	2	1	+	230
1938	–	69	135	63	59	11	10	1	+*	348
1939	+	8	109	89	28	28	5	1*	+*	268
1940	–	5	51	26	28	6	2*	+*	+*	118
1941	–	10	67	62	16	5*	1*	+*	+*	161
1942	–	17	123	50	19*	6*	2*	+*	+*	217
1943	–	94	360	186*	71*	23*	6*	1*	+*	741
1944	3	53	?	?	?	?	?	?	?	?

*Estimated.

1931 to 1943 over all age groups. Although subject to several sources of error which will be apparent from the discussions of previous sections, the totals for the year classes are considered to be roughly proportional to abundance. The results are illustrated in Fig. 5*B*.

It will be noted that three of the thirteen year classes have been exceptionally good—1931, 1933, and 1943, whereas at least two have been exceptionally poor—1936 and 1940. Of the remainder, special mention should be made of the 1938 year class which, although only of fair strength, was relatively more abundant than all others over the period 1934 to 1942.

From Figs. 5*A* and 5*B* a comparison may be made between the size of the spawning population (Index A) and the size of the resultant year class. A lack of close relationship is at once apparent: large year classes have not necessarily been produced by large spawnings, and small year classes have not necessarily been produced by small spawnings. For example, the exceptionally good 1931, 1933, and 1943 year classes were derived from a large, a medium, and a small spawning; the exceptionally poor 1936 and 1940 year classes were derived from a large and a medium spawning. The data even suggest an inverse relationship, large spawnings tending to produce small year classes, but analysis does not establish the statistical significance of the weak negative correlation which is obtained ($r = -0.30$; $P = 0.3$).

It seems likely that natural variation in the survival rate of immature fish, rather than fishing intensity, has been mainly responsible for the fluctuations in year class abundance which have taken place. Although a general downward trend in the abundance of the year classes from 1933 to 1942 might suggest a progressive decline due to the action of the fishery, the trend was halted, temporarily at least, by the appearance of the rich 1943 year class. The possibility that the trend is related to a cyclic phenomenon, with a periodicity of about five or ten years, must not be overlooked.

The abundance of the 1943 year class, the first rich brood to enter the fishery in several years, was mainly responsible for the increased availability of herring during the 1945-6 fishing season, when the quota of 25,000 tons was reached for the first time in several years. Although its ultimate magnitude has been estimated from its known strength at ages II and III only, its large abundance has since been confirmed.

Determination of the causes of fluctuation in the abundance of the year classes is one of the most important yet one of the most difficult problems of the investigation. Attempts have been made to correlate variation in year class abundance with variation in the physical environment in so far as measurements of the latter are available, but so far without success. Rounsefell (1930) found a positive correlation ($r_S = 0.73$; $P = 0.05$) between variation in average air temperature during the combined months of March to June, and variation in the strength of successive year classes (1921 to 1927) of herring at Prince William Sound, Alaska, *warmer* springs apparently resulting in year classes of greater abundance. As shown by a comparison of Figs. 5*B* and 5*C*, the latter including average air temperatures for the combined months of March to June at Estevan Point, this relationship does not hold for the west coast of Vancouver Island over the period 1931 to 1943. Rather, a negative correlation ($r = -0.55$; P = 0.05 is obtained which is even higher ($r = -0.66$; P = 0.025) when July air temperatures are used, suggesting that *colder* springs and summers result in year classes of greater abundance. In the face of this conflicting evidence, at the present time it can only be concluded that a relationship has not been proven, despite the statistical significance of both sets of data. It seems extremely unlikely that a linkage between temperature and year class abundance exists which would produce variation in one direction in Alaska and in the opposite direction in British Columbia. However, the co-variation found in British Columbia, which has been particularly noticeable since 1933, and which also exists between July *water* temperatures and year class abundance, will be watched with interest in future years. In the meantime it is idle to speculate as to its possible biological significance.

RELATIONSHIP BETWEEN EGG DEPOSITION, RECRUITMENT, AND MAXIMUM SUSTAINED YIELD

It has been shown in the previous section that during recent years there has been no close relationship between the size of the spawning stock (or relative number of eggs deposited) and the size of the resultant year class (or relative number of recruits to the fishable population). Big spawning stocks have produced small year classes and small spawning stocks have produced big year classes. This situation is further illustrated in Fig. 7 in which recruitment (relative abundance of the year classes—Table VII) is plotted against spawning potential (Index A—Table III). Attention is called to the random scattering of the plotted points.

As spawning potential decreases towards zero there must ultimately be a decrease in the number of recruits to the fishable stock. The type of average relationship which might exist is illustrated by the heavy broken line of Fig. 7, possible limits of dispersion being indicated by light broken lines (all lines drawn by eye). The curve is very similar to that postulated for fisheries populations in general by Kestevan (1947), but in the present case it is assumed to be asymptotic over the prevailing range of values. If this hypothetical relationship is generally correct, up to a point (X), on an average more spawners will produce more recruits to the fishable stock and hence larger year classes. Beyond that point it may be assumed that decreased survival from "population pressure" (overcrowding and consequent greater loss because of competition for food, incidence of disease, etc.) becomes a limiting factor to further increase in recruitment, thus keeping the population within bounds. Conversely, when a virgin population is fished, it may be assumed that population pressure is reduced and survival is increased. If a population did not exhibit this resilience, it would progressively decrease in abundance under the sustained action of a fishery, regardless of intensity. The spawning population at this point (*X*) may therefore be taken as the optimum for producing maximum sustained yield in numbers.

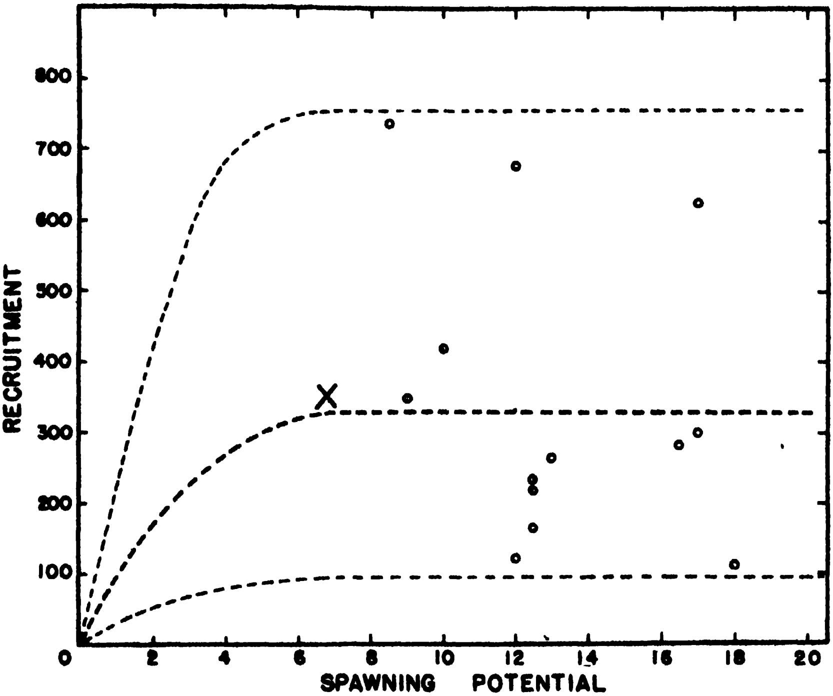


Figure 7.—Theoretical relationship between spawning potential (Index A) and recruitment (relative year class abundance).

It may be readily shown that in the fishable stock of herring gain in weight from growth of the main age groups contributing to the fishery (Table IV) is less than the loss from natural mortality, estimated from the age composition of virgin or nearly-virgin populations to be about 50 per cent per annum (Tester, MS). Therefore, in the words of Thompson and Bell (1934, p. 10), "the greatest yield from a given number of incoming young is produced by an intense fishery, although the catch per unit of gear is thereby sharply reduced" and "the intensity should be as great as is consistent with the maintenance of the supply of young and as is economically feasible in view of the fall in returns to the individual fishermen." Thus in the herring fishery, the spawning population at the point discussed above (X) also represents the optimum for producing maximum sustained yield in weight, although at this level of population abundance the catch per unit of gear may be reduced, and the population, depending on fewer age groups, may undergo more pronounced annual fluctuations in abundance.

Although the size of the spawning population which will result in maximum sustained catch is at present unknown, there is no good evidence that it has been reduced below the critical minimum by the level of fishing intensity of recent years, thus resulting in a progressive downward trend in the abundance of the fishable stock. However it will be realized that evidence of such a trend, if slight, would be difficult to obtain because of the large natural fluctuations in the abundance of successive year classes which take place.

PRACTICAL CONSIDERATIONS

A survey of catch statistics for the west coast of Vancouver Island herring fishery has not disclosed a progressive downward trend in catch for the sub-district as a whole since the start of intensive fishing about 1920. The catch over this period for Area 23 might be interpreted as indicating a local decline in abundance, but the extent of mixing is such that the run to Area 23 must be considered as one of a series of intergrading runs which, for practical purposes, are regarded as constituting one major population. Although there has been no pronounced decline in catch for the major population, there have been more violent fluctuations in recent seasons as compared with early years of the fishery. These must be expected under conditions of more intensive fishing.

In recent years, when quota restrictions were in force, the catch declined from a high to a low level and then increased again, even though fishing effort was maintained at a relatively high level over the period. The decline was due primarily to the absence of particularly abundant year classes for several years in succession. The recovery was due to the appearance of the rich 1943 year class which entered the fishery towards the end of the period. These fluctuations took place despite catch curtailment by quotas in the years of high abundance. There is no satisfactory evidence of a progressive decline in population abundance because of overfishing. However, proof of a decline would be very difficult to obtain because of the large natural fluctuations in abundance which take place.

It has been shown that, at the existing level of fishing intensity, there has been no close relationship between the size of the spawning population and the size of the resultant year class. However, apart from annual fluctuations, a general relationship must exist, for as the spawning stock progressively decreases towards zero, ultimately recruitment must also decrease. The average size of the spawning stock below which a progressive decrease in recruitment will occur, is regarded as the optimum for producing maximum sustained yield. As yet there is no satisfactory evidence that this optimum has been reached or exceeded.

In the face of this uncertainty it is questionable whether restriction of the catch by fixed quotas is justifiable. Grave doubts arise when particular cases are cited. For example, in one year, 1935-6, when the catch was limited to 25,000 tons, an excellent spawning resulted but it produced a very poor year class (1936); in another year, 1942-3, when the quota was not reached and fishing effort was increased by extending the length of the fishing season, a very poor spawning took place but it produced an excellent year class (1943). These doubts are of grave concern when it is realized that thousands of tons of fish and hundreds of thousands of dollars may have been wasted by unnecessary restrictions not only on the west coast of Vancouver Island in certain years, but also in other sub-districts where the catch has been more consistently curtailed by quota restrictions. It is therefore of very great economic importance to determine whether catch limitations, in addition to a prescribed date of closure, are necessary to prevent a progressive downward trend in population abundance and if so, whether a rigid quota system is the best that can be devised to attain the goal of maximum sustained catch.

RECENT APPROACH TO THE PROBLEM

In an attempt to determine by the most direct method whether or not quota restrictions are necessary, in 1946-7 it was agreed by representatives of the fishing industry, Dominion Department of Fisheries, provincial Department of Fisheries, and Fisheries Research Board of Canada to abandon catch quotas in the west coast of Vancouver Island sub-district for an indefinite number of years and to study the reaction of the population to the increased fishing effort which would probably result. The closing date of February 5 was retained to prevent fishing during the spawning period when, as already pointed out, the fish were in poor condition and formed compact sluggish schools which were particularly vulnerable to capture. It was also agreed that the population supplying the lower east coast of Vancouver Island sub-district should serve as a control under a fixed annual quota of 40,000 tons.

In embarking on this large-scale experiment, it was realized that a risk of overfishing was involved but that this risk must be taken if the question were to be answered within a reasonable period of time. It was pointed out that at a higher fishing intensity the catch might undergo more violent annual fluctuations; that a slight progressive downward trend in abundance due to fishing activity would be difficult to detect because of these fluctuations; and that, should a sharp decline occur which could reasonably be ascribed to fishing activity, drastic restrictions might be necessary to rehabilitate the population. In the latter event, it was hoped that sufficient scientific information would have been obtained to establish a more efficient system of management. On the other hand there was the possibility that even with a highly intensive fishery during the open season, a sufficiently large body of fish would enter inshore waters after the date of closure to provide an adequate spawning population, and that catch restrictions could be dispensed with, not only on the west coast of Vancouver Island, but also in other areas where they had been applied, and where in some cases they had been much more effective in limiting fishing effort.

When this programme was adopted in 1946-7, all phases of herring investigation on the west coast of Vancouver Island were intensified, including the collection of catch statistics, sampling of the commercial catches and spawning runs, tagging and tag recovery, and particularly spawning ground surveys which were undertaken by scientific investigators as well as by fisheries inspectors. In addition, a new phase of investigation was started, namely a study of the distribution, abundance, and growth of larval and young herring in an effort to determine the causes of fluctuation in the abundance of successive year classes.

The results for 1946-7 (Tester and Stevenson, 1947) and 1947-8 (not yet published) are encouraging and will be reviewed briefly in part.

In 1946-7 fishing effort increased considerably over that of the previous year. The catch amounted to 58,797 tons, which is more than double that of the previously existing limit of 25,000 tons. The season was closed on January 25 rather than on February 5, but as the catches had fallen off previous to closure and most of the boats had left or were planning to leave for more productive grounds, fishing effort was not restricted to any great degree. The catch was composed mostly of fish of the 1944 year class at age III and the 1943 year class at age IV, both of which year classes were of greater-than-average abundance. Despite the fact that few fish were left in inshore waters after the close of the fishing season, new influxes produced a spawning which was close to the average over the previous ten-year period (Index B was 65.5 compared with an average of 72.8).

In 1947-8 a high fishing effort was maintained. The catch amounted to 45,161 tons which is less than that of 1946-7 but still considerably above the previously existing limit of 25,000 tons. The catches fell off during the third week of January and by January 24 all seine boats had left the sub-district for more productive grounds. Although data on sampling and spawning ground surveys have not yet been fully analysed, the indications are that the 1945 year class at age III was well represented in the catches and that the spawning was equal to or greater than that of the preceding year.

In the lower east coast of Vancouver Island sub-district where catch restrictions are being retained, the quota of 40,000 tons was reached and the area was closed after a short season of intensive fishing in both 1946-7 and 1947-8.

The west coast of Vancouver Island experiment has been started during a period of high abundance, with two and possibly three successful year classes contributing to the catch. The previous attempt at management, the imposition of catch quotas, was also started during a comparable period of high abundance, 1935-6 and 1936-7. If the past history of the population can serve as a guide to the future, a decline in abundance primarily due to natural causes but accentuated by high fishing intensity may be expected. This decline, if it is experienced, should not be confused with a progressive decrease in abundance which might result from decreased spawning. To obtain the desired information on the effect of decreased spawning potential on recruitment, it is likely that the experiment will have to be continued over a period of at least ten years, during which time conviction as to the ultimate benefits of positive knowledge must outweigh fear of overfishing.

ACKNOWLEDGMENTS

It would be very difficult to enumerate the many individuals who have rendered assistance to the British Columbia herring investigation since its inception. It must suffice to say that we have been most fortunate in receiving splendid co-operation from the herring fishing industry, the herring fishermen, the Dominion Department of Fisheries, and the provincial Department of Fisheries. The industry has assisted in many ways, such as supplying vessels charter-free, making available plant records of landings, and allowing us to install tag recovery equipment in the herring canneries and reduction plants. The fishermen have voluntarily supplied us with daily catch records and other information pertaining to the herring fishery. The Department

of Fisheries has kept us posted on both fishing and spawning activity, has made available fisheries inspectors for herring spawning ground surveys, and has assisted in many other ways. The provincial Department of Fisheries has constantly stimulated interest in the investigation and has contributed financially to its cost in most years since 1929. To all of these organizations and to the many individuals connected with them, we wish to express our sincere thanks.

In particular we are indebted to Dr. R. E. Foerster, Director of the Pacific Biological Station, for general supervision, and to him and other members of the staff for advice, assistance, and criticism.

REFERENCES

Clemens, W. A., Hart, J. L., and Wilby, G. V. (1936). Analysis of stomach contents of fur seals taken off the west coast of Vancouver Island in April and May, 1935. Dominion Dept. Fish. (Ottawa): 1-20.

Hart, J. L. and Tester, A. L. (1937). The tagging of herring (*Clupea pallasii*) in British Columbia: methods, apparatus, insertions and recoveries during 1936-37. Rept. B.C. Prov. Fish. Dept., 1936. 55-67.

———— (1938). The tagging of herring (*Clupea pallasii*) in British Columbia: apparatus, insertions and recoveries during 1937-38. Rept. B.C. Prov. Fish. Dept., 1937: 64-90.

———— (1939). The tagging of herring (*Clupea pallasii*) in British Columbia: apparatus, insertions and recoveries during 1938-39. Rept. B.C. Prov. Fish. Dept., 1938: 51-78.

———— (1940). The tagging of herring (*Clupea pallasii*) in British Columbia: insertions and recoveries during 1939-40. Rept. B.C. Prov. Fish. Dept., 1939: 42-66.

Hart, J. L., Tester, A. L., and Boughton, R. V. (1942). The tagging of herring (*Clupea pallasii*) in British Columbia: apparatus, insertions and recoveries during 1941-42. Rept. B.C. Prov. Fish. Dept., 1941: 49-78.

Hart, J. L., Tester, A. L., and McHugh, J. L. (1941). The tagging of herring (*Clupea pallasii*) in British Columbia: insertions and recoveries during 1940-41. Rept. B.C. Prov. Fish. Dept., 1940: 47-74.

Kesteven, G. L. (1947). Population studies in fisheries biology. Nature, **159**: 10-13.

Petersen, C. G. J. (1922). On the stock of plaice and the plaice fisheries in different waters. Rept. Danish Biol. Sta., no. 29: 1-43.

Rounsefell, G. A. (1930). The existence and causes of dominant year classes in the Alaska herring; in Contributions to marine biology, pp. 260-70. Stanford University.

Tester, A. L. (1935). The herring fishery of British Columbia - past and present. Bull. Biol. Board of Canada, no. 47: 1-37.

———— (1936). Some results of the British Columbia herring investigation and their economic bearing. Rept. B.C. Prov. Fish. Dept., 1935: 74-80.

———— (1937a). Populations of herring (*Clupea pallasii*) in the coastal waters of British Columbia. J. Biol. Board of Canada, **3**: 108-144.

———— (1937b). The length and age composition of the herring (*Clupea pallasii*) in the coastal waters of British Columbia. J. Biol. Board of Canada, **3**: 145-168.

——— (1943). Use of the echo sounder to locate herring in British Columbia waters. Bull. Fish. Res. Board of Canada, no. 63: 1-21.

——— (1944). Tagging of herring (*Clupea pallasii*) in British Columbia: insertions and recoveries during 1943-44. Rept. B.C. Prov. Fish. Dept., 1943: 53-74.

——— (1945a). Tagging of herring (*Clupea pallasii*) in British Columbia: insertions and recoveries during 1944-45. Rept. B.C. Prov. Fish. Dept., 1944: 45-63.

——— (1945b). Catch statistics of the British Columbia herring fishery to 1943-44. Bull. Fish. Res. Board of Canada, no. 67: 1-47.

——— (1946). Tagging of herring (*Clupea pallasii*) in British Columbia: insertions and recoveries during 1945-46. Rept. B.C. Prov. Fish. Dept., 1945: 43-66.

TESTER, A. L. and BOUGHTON, R. V. (1943). Tagging of herring (*Clupea pallasii*) in British Columbia: apparatus, insertions and recoveries during 1942-43. Rept. B.C. Prov. Fish. Dept., 1942: 44-69.

TESTER, A. L. and STEVENSON, J. C. (1947). Results of the west coast of Vancouver Island herring investigation, 1946-47. Rept. B.C. Prov. Fish. Dept., 1946: 42-71.

THOMPSON, W. F. and BELL, F. H. (1934). Biological statistics of the Pacific halibut fishery. II. Effect of changes in intensity upon total yield and yield per unit of gear. Rept. Internat. Fish. Comm., no. 8: 1-49.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Experiments on Human Cutaneous Sensory Thresholds

By I. MACLAREN THOMPSON, F.R.S.C.,
JAMES C. LUCE, and L. W. DENNY[1]

INTRODUCTION

We anatomists study mostly the bodies of other people, which are, of course, outside our own skins. But if we essay to study the bodies *inside* our own skins, we encounter fascinating problems of more than anatomical significance, such as the localization of pain. If one of us hammers his thumb-nail instead of the metal nail at which he aimed, he feels pain in the injured thumb; it is correctly localized in an anatomical part of his body But the soldier with a painful amputation stump often localizes his pain just as precisely in his non-existent foot and toes; moreover, many pains of cardiac origin are referred down the left arm, and we have record of the pain of angina pectoris, developing years after amputation of the left arm, being referred to the non-existent limb just as clearly as to one that exists. Here we touch upon an anatomical aspect of the age-old question of the relationship between body and mind; it seems that what we are aware of is not our real bodies, but the concept or thought-model of our bodies that we have in our minds. This is known as the *body-image*, and it is in that image, not in our real bodies, that not only our normal sensations but also our aches and pains and many other symptoms are localized. To localize a sensation in an anatomical part of the body, what we must have is not the structure in reality but its image in mind.

The anatomical basis of the body-image consists partly of neurons in the post-central gyrus of the cerebral cortex, often called the cortical sensory area, and in corresponding parts of the thalamus; the cells chiefly responsible for awareness of each part of the body may be termed a cerebral "centre" for that part (hand, foot, etc.); details of the relationships between peripheral parts and their cerebral centres, and of the organization of the centres themselves, are current problems in dynamic anatomy (cf. Woodburne and Crosby, 1938; Woodburne, 1939; Adrian, 1947). There are analogous problems in connection with the efferent or motor apparatus.

---

[1] From the Departments of Anatomy of the Universities of California and Manitoba. This investigation was aided by grants from the Board of Research of the University of California, and from the University of California chapter of the Society of Sigma Xi, to whom appreciative acknowledgments are tendered.

The precise anatomical connotation of the term sensory "centre" for a part of the body awaits elucidation. There is good evidence for believing that the centres for the major sub-divisions of the body are largely discrete; for example the foot is represented near the upper end of the post-central gyrus, the head near its lower end; this curious inversion in the cortical sensory area seems to be associated with the dominance of the visual sense in man and the well-known inversion of the retinal image, but that cannot be discussed here. Now though adjacent parts of the body (e.g., leg and foot) are separate, there is evidence (Penfield and Boldrey, 1937) that their sensory centres partly "overlap," as we say, though that term seems to be misapplied to what is probably really intermingling of neurones rather than true overlapping or imbrication.

It has been suggested that visual dominance has influenced the evolution of the cortical sensory area. But almost the only structure in our own bodies that we see is the skin; details of the central representation of the skin are therefore of special interest. The total cutaneous area being so much larger than the thalamic and the cortical sensory areas, "point-to-point" representation is out of the question; convergence upon final common pathways seems to lead to "area-to-point" representation (Woolsey, Marshall, and Bard, 1942), the extent of the cutaneous area represented at a cortical "point" varying inversely with acuteness of perception and hence precision of anatomical localization, as illustrated by the homunculus of Penfield and Boldrey (1937).

As the experiments herein reported were performed upon limb nerves, what follows refers to those. Each main limb nerve supplies a considerable area of skin (Thompson, Inman, and Brownfield, 1934), and adjacent nerve territories "overlap," though actually the terminal twigs of the two nerves intermingle and intertwine rather than literally overlap. Thus the *total* area of skin supplied by a specified nerve comprises (*a*) a central *clear* area supplied by that nerve only, being clear of all others, and (*b*) peripheral *overlap* areas, supplied, in addition to the nerve in question, by one or two others. Since the fibres subserving the several kinds of sensation (touch, pain, etc.) intermingle to varying extents, the sizes of the clear and of the overlap areas vary somewhat for the different sensations, the clear and overlap areas varying inversely, of course. If a nerve is severed, or completely blocked with a local anaesthetic, sensibility is altered over an area of skin corresponding approximately to the total area supplied by the nerve. But sensibility is completely lost (thresholds elevated beyond estimation) only in the central clear area. In the peripheral overlap areas sensation is modified but not completely lost; thresholds are elevated differentially, and abnormal qualities of sensation appear that cannot be discussed here. These phenomena are caused by a peripheral lesion; and it seems reasonable to attribute the sensibility remaining in the overlap areas to the intact afferent pathways from those areas through unaffected nerves. Everyday experience gives us the impression that sensory thresholds do not differ noticeably in the doubly-innervated overlap areas and in the singly-innervated clear areas; but in the presence of the type of peripheral lesion described, the intact afferent pathway from an overlap area reveals itself by the generally lower thresholds therein than in the clear area of the affected nerve.

In 1933 Thompson and Inman observed that if a human nerve trunk is stimulated through the skin by an appropriate alternating current, tingling is felt in the total cutaneous area supplied through that nerve; and throughout the area thresholds are elevated differentially, in the radial nerve area pressure being raised most, then touch, pain, and temperature much less (Thompson, 1933; Thompson, Banks, Barron, Fratis, and Mattison, 1934). Here there was some superficial resemblance to local anaesthesia; but whereas local anaesthesia results from a *peripheral* nerve lesion (blocking or severance of nerve fibres), Thompson (1935, 1943) has adduced experimental evidence proving that the elevation of cutaneous sensory thresholds during such electrical stimulation of a nerve is not local anaesthesia caused peripherally, but *masking*, a central phenomenon presumably involving the cerebral sensory centres; furthermore, Marshall, Woolsey, and Bard (1937) have made a promising experimental beginning at elucidating the central mechanism of the masking of cutaneous sensations. The mechanism of masking being central, we wondered how the degree of masking by a certain alternating current in the clear area supplied by the nerve under stimulation would compare with that in an overlap area supplied, in addition, by another nerve; this is the investigation herein reported and discussed. An essential part of this research was, of course, to ascertain the normal thresholds at the same places.

EXPERIMENTS

By the method of Thompson and Inman (1933), described fully by Thompson, Inman, and Brownfield (1934), the boundaries of the areas supplied for touch by each of the nerves specified below were mapped, on the number of subjects stated, sufficiently to identify the areas supplied, as follows: radial, dorsal branch of ulnar, radio-ulnar overlap; medial cutaneous nerve of forearm, lateral cutaneous nerve of forearm, medial cutaneous-lateral cutaneous overlap; dorsal cutaneous nerve of forearm, lateral cutaneous-dorsal cutaneous overlap. By a simple adaptation of the method of Thompson, Banks, Barron, Fratis, and Mattison (1934) for estimating the threshold for pressure, the areas supplied for pressure by the same nerves were also mapped on the same limbs. Near the centre for each clear area we marked two convenient points, *A* and *B*, and near the centre of each overlap area two others, *C* and *D*· thus *A* and *B* were in an area supplied both for touch and for pressure by a single nerve, whereas *C* and *D* were within an area supplied both for touch and for pressure by two nerves. In selecting these points we tried to avoid such things as superficial veins; hairs were shaved off, and their roots avoided as far as possible.

The apparatus and techniques used for generating and applying the alternating currents and for gauging the sensory thresholds were those described and illustrated in detail by Thompson, Banks, Barron, Fratis, and Mattison (1934); the current frequencies were of the order of 200 per second, their strengths falling short of causing distress (e.g. 9 volts, 2.5 ma.).

The electrode having been adjusted over the nerve to be stimulated, the normal threshold for touch at point *A* was measured; the current was introduced and recorded, and the threshold for touch at *A* again gauged; the current was withdrawn and the normal tactual threshold at *B* was taken; the *same* current was reintroduced and the tactual threshold at *B* again measured. This procedure was repeated at *A* and *B*, still using the same current but now gauging the thresholds for pressure. Then points *C* and *D* were tested similarly, always stimulating the same nerve with the same current. When the threshold rise in a clear area was compared with that in one of the overlap areas of that nerve, and with that in another, different *A* and *B* points were used in the two instances; and when the threshold rise in an overlap area was compared with that in the clear area of one of its nerves, and with that in the clear area of the other, different *C* and *D* points were used in the two instances.

The rise in threshold under the influence of the current was measured from the normal threshold for the same sensation at the same point and (to validate comparisons) was expressed as a percentage of that normal. Thus for each nerve experimented upon we obtained a pair of values referring to touch at each of the four places, *A*, *B*, *C*, and *D*: each pair comprised (*a*) the normal threshold and (*b*) the percentage elevation of threshold under the influence of the alternating current; and we obtained a similar set of values for pressure. For comparison of values in clear and in overlap areas, those obtained at *C* were always subtracted algebraically from those at *A*, and those at *D* from those at *B*. The following summary indicates (1) the nerves experimented upon, (2) the number of subjects, and (3) the values obtained.

Radial nerve: three subjects; from each 2 pairs of values for touch and 2 pairs for pressure in clear radial area; the same in radio-ulnar overlap; hence 4 clear-overlap differences (2 normal and 2 under stimulation) for touch and 4 corresponding differences for pressure.

Dorsal branch of ulnar nerve: three subjects; a similar set of data respecting clear ulnar area and radio-ulnar overlap.

Medial cutaneous nerve of forearm: two subjects; from each 4 pairs of values for touch[2] and 4 pairs for pressure in clear medial cutaneous area; 2 pairs of values for touch and 2 pairs for pressure in medial cutaneous-lateral cutaneous overlap; 2 pairs of values for touch and 2 pairs for pressure in medial cutaneous-dorsal cutaneous overlap; hence 4 clear-overlap differences (2 normal and 2 under stimulation) for touch and 4 corresponding differences for pressure in respect of medial cutaneous-lateral cutaneous overlap; and a similar set of differences in respect of medial cutaneous-dorsal cutaneous overlap.

Lateral cutaneous nerve of forearm: two subjects; from each 2 pairs of values for touch and 2 pairs for pressure in clear lateral cutaneous area; 2 pairs of values for touch and 2 pairs for pressure in medial cutaneous-lateral cutaneous overlap; and hence 4 clear-overlap differences (2 normal and 2 under stimulation) for touch and a corresponding 4 for pressure; from one subject a similar set of values and of differences in respect of the lateral cutaneous-dorsal cutaneous overlap.

Dorsal cutaneous nerve of forearm: one subject; 2 pairs of values for touch and 2 pairs for pressure in clear dorsal cutaneous area; 2 pairs of values for touch and 2 pairs for pressure in lateral cutaneous-dorsal cutaneous overlap; hence 4 clear-overlap differences (2 normal and 2 under stimulation) for touch and a corresponding 4 for pressure.

---

[2] One of the readings of the tactual threshold under electrical stimulation was so characteristic of a technical blunder that it was omitted from the computations.

These experiments, involving five nerves and three subjects, yielded a total of 223 observed threshold readings, leading to the following results.

1. *Difference between the normal threshold for touch in a clear area and that in an overlap area supplied by the same nerve:* 28 values; mean, 3.6 mg. (higher in overlap areas). But 3.6 mg. is so well within the experimental error of our technique (see Thompson, Banks, Barron, Fratis and Mattison, 1934) that so small a difference must be considered without significance.

2. *Difference between the normal threshold for pressure in a clear area and that in an overlap area supplied by the same nerve:* 28 values; mean, 0.36 gm. (higher in clear areas). For the same reason as with touch, no significance may be attributed to this difference.

3. *Difference between the percentage elevation of the threshold for touch produced by the same current in a clear area and in an overlap area supplied by the same nerve:* 27 values; mean, 1,112 per cent (higher in clear areas); t, 2.815; $P < 0.01$; highly significant.

4. *Difference between the percentage elevation of the threshold for pressure produced by the same current in a clear area and in an overlap area supplied by the same nerve:* 28 values; mean, 1,495 per cent (higher in clear areas); t, 3.286; $P < 0.01$; highly significant.

5. *Difference between the mean excess (1495) of the percentage elevation of the pressural threshold in clear areas over its percentage elevation in overlap areas and the corresponding mean excess (1112) for touch:* 383 per cent (greater in the case of pressure); t, 0.635; $0.5 < P < 0.6$; insignificant.

6. *Difference between the excess of the percentage elevation of the pressural threshold over that for touch in a clear area and the corresponding excess in an overlap area supplied by the same nerve:* 27 values; these were so obviously inconsistent in themselves that a mean was not even computed.

These results reveal no significant differences between the normal thresholds for touch and for pressure in clear and in overlap areas; this accords with ordinary experience.

But our experiments did disclose, on the average, roughly an eleven-fold greater elevation by an alternating current applied to a nerve on the part of the threshold for touch in the clear area of that nerve than the corresponding elevation in an overlap area supplied also by another unstimulated nerve. In the case of pressure the elevation in the clear areas was on the average about fifteen times as great as in the overlap areas. But the differences between the percentage elevations of the thresholds for pressure and for touch were probably fortuitous.

DISCUSSION

Since there is no significant difference between the thresholds in clear and in overlap areas under normal circumstances, whereas a marked difference accompanies stimulation of a nerve by a suitable alternating current, the latter appears responsible for the difference. We interpret our results in the light of Thompson's (1935, 1943) experimental demonstration that the rise in thresholds in the cutaneous area supplied by a nerve under stimulation by such an alternating current is *not* local anaesthesia of peripheral origin, but is a *central* phenomenon termed *masking*, produced by bombarding the sensorium with nerve impulses ascending from the electrode along the nerve and the spinal cord to the brain; we therefore interpret our results in terms of cerebral *centres*, doubtless both thalamic and cortical. By the centre for a nerve and its cutaneous area we mean those neurons that ordinarily receive impulses from the specific cutaneous area through the nerve in question (e.g. the radial). Such centres need not be grossly discrete (like the cranial nerve nuclei); indeed, cerebral microscopic anatomy tells us that they are more likely continuous (like the segments of the spinal cord). Recollection that the spinal cord segments are defined according to their peripheral nerve connections shows that defining cerebral centres on a similar basis conforms to an accepted neuro-anatomical custom; it conforms also to the work of Woolsey, Marshall, and Bard (1942) on cortical dermatomal fields. There *may* be various centres for the varieties of sensation, but we present no evidence bearing on that question.

Since masking is a central phenomenon, the different degrees of masking in clear and in overlap areas bespeak the existence of a centre (as defined above) for each clear area and one for each overlap area, the anatomical difference being that the afferent pathways converging upon the centre for a clear area traverse a single nerve trunk, whereas those converging upon the centre for an overlap area course through two peripheral nerves. A "clear" centre belongs to one nerve, whereas an "overlap" centre may be assigned with equal justification to either of its nerves, for our experiments show that its activity may be modified by stimulating either of them. We might speak of a total centre for each nerve and its total cutaneous area, subdivided into a clear centre and overlap centres as defined above. In a cerebral overlap centre there is intermingling of neurones conveying impulses *from* two or more nerve trunks, just as in a cutaneous overlap area there is intermingling of neurones transmitting impulses *through* two or more nerve trunks.' Briefly, the central arrangement corresponds to that at the periphery.[3] This inkling of part of the organization of the anatomical basis of the body image helps us to understand a little how that image is as accurate as it is with respect to the visible exterior of the body, and how accurately symptoms (such as pains) of superficial origin can be localized, in contrast to those arising in the inner parts that do not appear in the body-image of even the most learned anatomist.

Detailed discussion of the fit of this hypothesis to the facts seems unnecessary, but the following remarks are offered. Unlike nerve sections, where all the fibres are cut, there was no interruption of peripheral pathways; indeed, it is virtually certain that some fibres in the nerve were not even stimulated, but remained available to transmit impulses from their cutaneous endings both in the clear and in the overlap areas; for, contrary to what happens after nerve section, sensation can usually be evoked from a masked area (clear or overlap) if the stimulus to the end-organs be sufficiently strong (cf. Thompson, Banks, Barron, Fratis, and Mattison, 1934). In addition, of course, impulses from endings in overlap areas ascended freely along unstimulated nerves. If the pathways from clear and from overlap areas converged upon a single undifferentiated centre, our observed threshold *differences* could scarcely have arisen, nor could an overlap area be masked equally readily by stimulating either of its two nerves.

Having thus shown that peripheral nerve intermingling is probably repeated by the corresponding centres responsible for localizing sensations, we may point out a suggestive relationship between accuracy of localization and extent of intermingling or overlapping. A measure of accuracy of tactual localization is the liminal distance for two-point discrimination; Sherrington (1900) quotes the following averages: palm, 11.3 mm., forearm, 40.6 mm. By the electrical method of Thompson and Inman (1933), Thompson, Inman, and Brownfield (1934) measured the maximum linear overlaps between adjacent cutaneous nerve areas (tactual) in sixty-five living upper limbs; since the measurements appeared to follow a logarithmically normal distribution, the geometric mean was a better representative value for each group than the arithmetic mean; the maximum linear overlap between the median and the ulnar nerves in the palm averaged (geometrically) 13.8 mm., whereas the corresponding figure for the overlap between the lateral and the dorsal cutaneous nerves of the forearm was 29.2 mm. Comparison of the two pairs of figures illustrates the point under discussion: the extent of intermingling or overlapping varies directly with the degree of area-to-point convergence, and inversely with precision of anatomical localization, at least for touch.

---

[3] We have not thought it necessary to refer to the so-called "second" somatic area recently described in certain animals (cf. Woolsey, 1947), because, so far as we know, it has not yet been discovered in man, the subject of our research.

Our results are in line with the general principles of Woodburne (1939) concerning the anatomical basis of localization.

Mention must be made of the interesting work of Woolsey, Marshall, and Bard (1942) on overlapping of the cortical fields for the dermatomes, which of course overlap peripherally as do the cutaneous nerve areas. They think "that cortical overlaps merely reflect the overlapping cutaneous distributions of dorsal root fibres," and we could make a corresponding statement concerning cutaneous nerve areas and their centres. That independent investigations by methods so very different should lead to such closely complementary conclusions tends to consolidate the advances made by both groups.

A final word concerning the sensory results of peripheral nerve injuries, upon which this research has some bearing. By stimulating peripheral nerves we experimented upon the brain. After nerve severance there are peculiar abnormalities of sensation that are difficult to explain satisfactorily on a purely peripheral basis, but bear sufficient resemblance to the sensory abnormalities following certain cerebral lesions to lead such acute observers as Head and Stopford to suggest a central basis for them, even though the primary injury is peripheral. That our experiments point in the same general direction seems clear. But that, as Kipling says, is another story.

SUMMARY

1. Experiments are reported involving five nerves and three human subjects; the nerves were stimulated by suitable alternating currents, and the resulting masking of the sensations of touch and of pressure were recorded (*a*) in the clear area supplied only by the nerve under stimulation, and (*b*) in the overlap areas supplied in addition by other nerves. In all, 223 threshold readings were obtained.

2. Differences (twenty-eight in number) between the thresholds for touch and for pressure in clear and in overlap areas were insignificant under ordinary circumstances.

3. When a nerve was under stimulation by a suitable alternating current, the difference between the percentage elevation of the tactual threshold in the clear area and in the overlap areas averaged 1,112 per cent (27 values); the corresponding difference for pressure averaged 1,495 per cent (28 values).

4. This is interpreted as indicating that the arrangement of the sensory centres corresponds to that of the peripheral nerve distribution, pathways from clear areas going via single nerves to clear centres and pathways from overlap areas traversing two nerves to overlap centres.

5. The bearing of this research on the anatomical basis of the body image, the anatomical localization of sensations, the central representation of dermatomes, and the sensory results of nerve severance are discussed briefly.

REFERENCES

ADRIAN, E. D. (1947). The physical background of perception. Oxford, Clarendon Press.

MARSHALL, W. H., WOOLSEY, C. N., and BARD, P. (1937). Cortical representation of tactile sensibility as indicated by cortical potentials. Science, **85**: 388-90.

PENFIELD, W. and BOLDREY, E. (1937). Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain, **60**: 389-443.

SHERRINGTON, C. S. (1900). Cutaneous sensations; in Schäfer's Text-book of physiology, vol. II, pp. 920-1001. Edinburgh & London, Young J. Pentland.

THOMPSON, I. M. (1933). The effects of alternating currents upon cutaneous sensory thresholds. Science, **78**: 268-9.

—— (1935). Sensory masking—a peripheral or a central phenomenon? Science, **82**: 221.

—— (1943). An experimental anatomical study of sensory masking; in Essays in biology in honor of Herbert M. Evans, pp. 555-9. University of California Press.

THOMPSON, I. M., BANKS, G. F., BARRON, A., FRATIS, A. M., Jr., and MATTISON, B. F. (1934). Differential elevation of cutaneous sensory thresholds by alternating currents applied to a nerve. Univ. Calif. Publ. in Anat., **1**: 167-194.

THOMPSON, I. M. and INMAN, V. T. (1933). A method of outlining cutaneous nerve areas. Science, **77**: 216-7.

THOMPSON, I. M., INMAN, V. T., and BROWNFIELD, B. (1934). On the cutaneous nerve areas of the forearm and hand. Univ. Calif. Publ. in Anat., **1**: 195-236.

WOODBURNE, R. T. (1939). Certain phylogenetic anatomical relations of localising significance for the mammalian central nervous system. J. Comp. Neur., **71**: 215-257.

WOODBURNE, R. T. and CROSBY, E. C. (1938). The basis of cortical localisation. Anat. Rec., **70**: 84.

WOOLSEY, C. N. (1947). The somatic functions of the central nervous system. Ann. Rev. Physiol., **9**: 525-552.

WOOLSEY, C. N., MARSHALL, W. H., and BARD, P. (1942). Representation of cutaneous tactile sensibility in the cerebral cortex of the monkey as indicated by evoked potentials. Bull. Johns Hopkins Hosp., **70**: 399-441.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLII : SERIES III : MAY, 1948
SECTION FIVE

Distribution of Micro-organisms in the Rhizosphere of a Root System[1]

By M. I. TIMONIN and A. G. LOCHHEAD, F.R.S.C.

HILTNER (1904) was the first to observe that soil in the vicinity of the root of the growing plant contained a considerably denser microbial population than was found elsewhere in the same soil. This portion of the soil he called the *rhizosphere.* Various workers have confirmed Hiltner's findings and it is now established that the plant may exert a marked effect on the numbers and types of micro-organisms in the zone influenced by the roots. However, there is little information about the rhizosphere effect on microbial activity at different parts of the same root system, which was the object of the present investigation, and for which purpose the tobacco plant was chosen.

EXPERIMENTAL

The plant studied was taken from the experimental plots of the Central Experimental Farm when the crop was at the flowering stage. To obtain samples, a trench, 2′ × 5′ × 2′, was dug so that the base of the stem was situated at the centre of one of the long sides of the trench; thus a vertical section of the entire root system was exposed. The distribution of roots was then mapped to scale (Fig. 1) and six samples, located at different points of the root system, were taken for microbiological analysis. Each sample consisted of a five-inch cube of the soil, the base of the plant being situated at the centre of one side of the first sample (*A*-1). Each sample was placed in a separate container and taken to the laboratory for analysis.

The procedure for obtaining rhizosphere soil was similar to that previously described (Timonin, 1939). The numbers of different micro-organisms were estimated by plating or dilution methods.

---

[1] Contribution no. 260 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

Nutrient agar and soil extract agar of pH 6.8-7.0 and 3.8-4.0 were used for total counts of bacteria and fungi respectively, spore-forming bacteria being estimated on plates poured from dilutions after heating to 80° C. for ten minutes. Casein hydrolyzing bacteria were counted on milk agar plates.

The other groups of organisms were determined by the dilution method with appropriate selective liquid media. Cellulose decomposing bacteria were estimated in Omeliansky's medium, ammonifying organisms in gelatin and peptone solution, nitrifying bacteria in Winogradsky's medium, and denitrifying bacteria in Giltay's solution. Estimation of algae was made with the following medium: $Ca_3(PO_4)_2$ 0.5 gm.; K_2HPO_4 0.05 gm.; KCl 0.15 gm.; $MgSO_4$ 0.15 gm.; $NaHCO_3$ 0.5 gm.; $Ca(NO_3)_2$ 1.0 gm.; $FeCl_3$ trace; tap water 1000 ml. For protozoa, hay infusion medium was used.

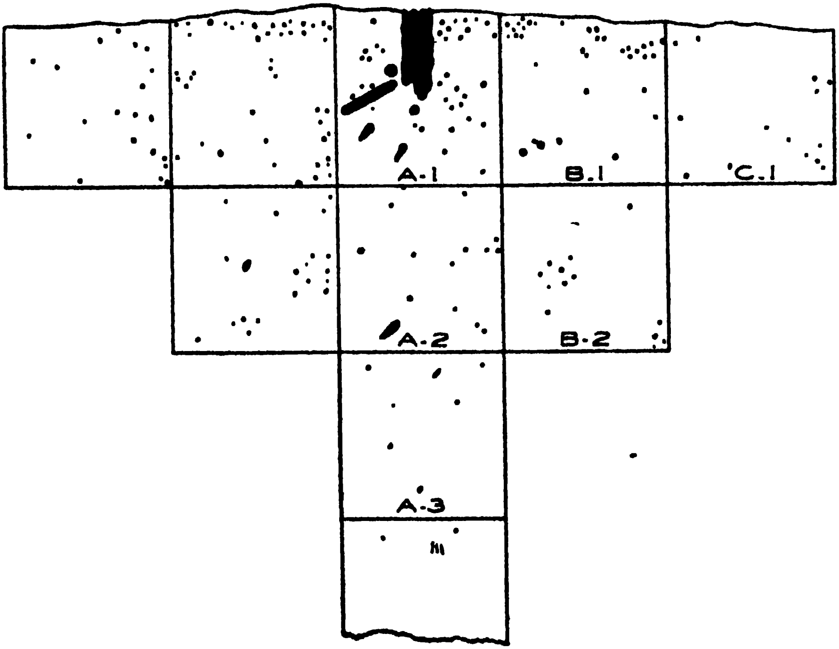


FIGURE 1.—Profile of tobacco plant root system.

For the classification of the rhizosphere bacteria according to their nutritional requirements, colonies were systematically picked from soil extract agar plates (approximately forty-five per plate) so that all on a sector were taken, and transferred to soil extract semi-solid medium. The nutritional grouping of the isolates was then carried out by the method of Lochhead and Chase (1943), which involved a determination of the growth response in seven media of increasing complexity, ranging from a simple basal medium to those containing in addition, amino acids, growth factors, amino acids plus growth factors, or the more complex ingredients of yeast extract, soil extract, or both.

DISTRIBUTION OF ROOT SYSTEM IN THE SOIL

The different points of the root system selected for the microbiological analyses are illustrated in Fig. 1. It is seen that the bulk of the root system is located in the upper five-inch layer of soil, the heavy lateral roots being concentrated in the centre and the two adjacent blocks. With increasing distance from the base of the stem, vertically or horizontally, the weight of root per block gradually declined.

TABLE I

INCIDENCE OF DIFFERENT MICRO-ORGANISMS
(per gram of rhizosphere soil)

	2½″	7½″	12½″
Bacteria—soil ex. ag.	1,050,000,000	1,495,000,000	1,200,000,000
" —nutrient ag.	1,350,000,000	930,000,000	495,000,000
Fungi	550,000	390,000	550,000
Algae	67,000	240,000	52,000
Protozoa	70	50	520
5″			
Bacteria—soil ex. ag.	1,420,000,000	740,000,000	
" —nutrient ag.	420,000,000	370,000,000	
Fungi	10,300	37,500	
Algae	6,200	7,500	
Protozoa	0	0	
10″			
Bacteria—soil ex. ag.	27,000,000		
" —nutrient ag.	5,500,000		
Fungi	4,000		
Algae	0		
Protozoa	0		
15″			

MICROBIAL POPULATION OF RHIZOSPHERE SOIL

Quantitative

The data summarized in Table I indicate that the density of the microbial population in the rhizosphere soil tends to decrease with increasing distance in horizontal, and more particularly in vertical, direction from the base of the stem. The bacterial population in the sample located at a depth of ten to fifteen inches was only 1.4 per cent of that of the sample containing the base of the plant. Fungi likewise showed a numerical decrease with increasing depth. Algae also showed the same pattern of decrease, while protozoa was recovered only from the surface samples of the rhizosphere soil.

Qualitative

As indicated in Table II, spore forming bacteria decreased numerically with increasing depth of sampling. However, in relation to

TABLE II

INCIDENCE OF VARIOUS GROUPS OF BACTERIA
(per gram of rhizosphere soil)

	2½"	7½"	12½"
Spore formers	3,500,000	4,400,000	3,300,000
Casein hydrolyzing	205,000,000	103,000,000	24,000,000
Cellulose decomposing	70,000	48,000	600
Ammonifying	1,350,000,000	950,000,000	250,000,000
Nitrifying	340,000	240,000	260,000
Denitrifying	340,000,000	48,000,000	5,200,000
5"			
Spore formers	3,400,000	2,300,000	
Casein hydrolyzing	28,000,000	26,000,000	
Cellulose decomposing	7,000	800	
Ammonifying	310,000,000	370,000,000	
Nitrifying	75,000	800	
Denitrifying	6,200,000	3,800,000	
10"			
Spore formers	200,000		
Casein hydrolyzing	100,000		
Cellulose decomposing	200		
Ammonifying	8,000,000		
Nitrifying	0		
Denitrifying	0		
15"			

the total bacteria population of each sample, the results indicate that the proportion of spore formers increases with depth, this group of organisms comprising 0.18 per cent, 0.24 per cent, and 0.74 per cent of the total in blocks *A*-1, *A*-2, and *A*-3, respectively.

The various physiological groups of bacteria decreased sharply in number with depth, and in most cases, though less markedly, with horizontal distance from the base of the stem. In the upper section of the soil nitrifying organisms showed little change with distance from the stem. On the other hand, cellulose decomposing bacteria were much more numerous in the rhizosphere at the base of the stem than in that of the younger portion of the root system further removed in a lateral direction, the count per gram of rhizosphere soil in *C*-1 being less than 1 per cent of that in *A*-1. Proteolytic and denitrifying forms also declined sharply in both horizontal and vertical directions.

TABLE III

RELATIVE INCIDENCE OF DIFFERENT NUTRITIONAL GROUPS OF BACTERIA

	2½"	7½"	12½"
I Basal medium	34.1 per cent	7.4 per cent	18.2 per cent
II Amino acids	17.1	44.4	36.3
III Growth factors	4.9	3.7	9.1
IV Amino acids + growth factors	7.3	3.7	3.0
V Yeast extract	34.1	25.9	24.2
VI Soil extract	2.5	11.1	0.0
VII Yeast + soil extract	0.0	0.0	3.0
5"			
I Basal medium	14.3	26.8	
II Amino acids	5.7	14.6	
III Growth factors	0.0	12.2	
IV Amino acid + growth factors	2.9	2.4	
V Yeast extract	11.4	36.5	
VI Soil extract	0.0	0.0	
VII Yeast + soil extract	62.8	4.9	
10"			
I Basal medium	7.5		
II Amino acids	15.0		
III Growth factors	7.5		
IV Amino acids + growth factors	2.5		
V Yeast extract	27.5		
VI Soil extract	7.5		
VII Yeast + soil extract	12.5		
15"			

Incidence of Different Nutritional Groups

The relative incidence of the various nutritional groups of bacteria is summarized in Table III. Previous work has shown that plant growth, as far as the upper layers of the soil are concerned, normally results in a preferential stimulation of organisms capable of maximum growth in the basal medium (Group I) and of those requiring amino acids (Group II), and depresses, relatively, those requiring the more complex ingredients of yeast and soil extract (Group VII). It is noted from Table III that the rhizosphere effect varies in different sections of the root system, and that bacteria of Group I are proportionately more abundant in the rhizosphere near the base of the stem than in surface sections farther removed, while those responding to amino acids are relatively more numerous in the sections farther from the base. In vertical direction from the base of the stem, bacteria with simplest requirements tend to become relatively less abundant.

DISCUSSION

Although considerable information has been published on the subject of the rhizosphere effect of the growing plant on the abundance and activity of soil micro-organisms, no previous studies appear to have been reported on the rhizosphere effect of different portions of the root system of the same plant. Though a number of workers (e.g. Starkey, 1931) have studied the microbial population at different distances from the main root of the plant, there is no evidence that they were dealing only with rhizosphere soil, as was the case in this investigation. While Starkey's work showed that bacterial numbers decline sharply in soil with increasing distance from the plant, the present findings show that in the rhizosphere of the same root system the effect varies with the location of the root portion.

It will be generally conceded that micro-organisms will develop to the greatest extent where environmental conditions and food supply are most favourable. The region of greatest root activity, where transfer of plant nutrients and of excretory products is highest, might be expected to be the terminal portions of the roots and particularly the root hair zone. In such zones certain groups of micro-organisms might be expected to be stimulated, particularly in the upper layers of soil, though near the older root portions (*A*-1) different types of organisms engaged in decomposing sloughed-off portions of root material would find a suitable environment for development. These views are supported by the preferential stimulation of bacteria responding to amino acids in the younger portions of the surface rhizosphere and the occurrence of much higher numbers of cellulose decomposing and proteolytic forms near the base of the stem.

REFERENCES

Hiltner, L. (1904). Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngung und Brache. Arb. deut. landw. Ges., **98**: 59-78.

Lochhead, A. G. and Chase, F. E. (1943). Qualitative studies of soil micro-organisms. V. Nutritional requirements of the predominant bacterial flora. Soil Sci., **55**: 185-95.

Starkey, R. L. (1931). Some influences of the development of higher plants upon the microorganisms in the soil. IV. Influence of proximity to roots on abundance and activity of microorganisms. Soil Sci., **32**: 367-93.

Timonin, M. I. (1939). The interaction of soil micro-organisms and higher plants. Thesis, Rutgers University.

TABLE OF CONTENTS

Observations on the Flora of the Southern Rocky Mountain Trench in British Columbia. By J. W. EASTHAM 1

L'Action bactériostatique du glycocolle et les besoins du rat en pyridoxine. Par EDOUARD PAGÉ et ROSAIRE GINGRAS . . . 11

An Experimental Anatomical Study of the Feeling of Pressure. By I. MACLAREN THOMPSON, F.R.S.C., L. W. DENNY, H. S. KIMBALL, and J. C. LUCE 21

Some Problems in the Cytogenetics of Cereals: A Historical Review. Flavelle Lecture, by W. P. THOMPSON, F.R.S.C. 35

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIII : SERIES III : JUNE, 1949
SECTION FIVE

Observations on the Flora of the Southern Rocky Mountain Trench in British Columbia

By J. W. EASTHAM
Presented by H. T. GÜSSOW, F.R.S.C.

THIS paper deals with that portion of the Rocky Mountain Trench which lies between the Rocky and Selkirk Mountains, from the International Boundary north to Golden on the main line of the Canadian Pacific Railway. Only the vegetation below about 3500 ft. elevation was studied, and on this basis the above stretch of the Trench falls fairly definitely into three sections which may be designated Southern, Middle, and Northern, available soil moisture being the governing factor in determining the type of vegetation. Special attention was given to the southern section, where the valley floor is widest and conditions most varied. In this area, also, far-reaching plans are being made for dams on the Kootenay River and its tributaries, with the objectives of flood-control and power-development. If these are carried through they will also provide water to irrigate many thousands of acres, and change the agricultural development of the area, which is now mainly stock-range. For this reason, and also because the botany of this region has been much neglected, the writer believes that his observations, made in the summers of 1947 and 1948, may be of interest and provide a record of conditions liable to change greatly in the near future.

I. SOUTHERN SECTION

The area from the Montana Boundary, north to and including Canal Flats, bounded on the west by Cranbrook and on the east by Elko, forms a fairly definite vegetation zone. The altitude of the valley-floor is indicated by that of the following points: Canal Flats, 2,653 feet; Cranbrook, 3,013 feet; Elko, 3,000 feet; Newgate, almost on the International Boundary, 2,800 feet.

The surface is much broken up with rocky knolls and ridges, river and stream valleys, and some deep canyons, giving a great variety of drainage and exposure, with consequent changes in the composition of the vegetation.

Precipitation, as shown in the following table, is intermediate between that of Vernon and Kelowna, at the northern extremity of the Okanagan Dry Belt.

	Alt. Ft.	April In	May In	June In	July In	Aug. In.	Sept. In.	6 mos. In.	12 mos. In.
Cranbrook	3,013	0.67	1.24	1.81	1.13	0.94	1.14	6.93	14.16
Newgate	2,800	0.78	1.25	1.60	0.85	0.82	0.89	6.19	13.52
Kelowna	1,200	0.62	0.92	1.10	0.77	0.79	1.12	5.32	12.18
Vernon	1,383	0.77	1.16	1.74	0.98	1.14	1.28	7.07	15.60

The vegetation is that of the upper and moister levels of the Arid Transition Zone, modified perhaps by much severer winters than in the Okanagan and Kamloops areas. Sage-brush (*Artemisia tridentata* Nutt.) and cactus (*Opuntia* sp.), conspicuous plants of the Western Dry Belt, do not occur. Rabbit-brush (*Chrysothamnus nauseosus* (Pall.) Britt.) and balsam-root (*Balsamorhiza sagittata* Nutt.) are found only here and there on hot, dry, usually southern, exposures.

Although, as mentioned above, there are local variations in the character of the vegetation, there is little evidence of zonation, either regional or altitudinal, within our terms of reference, except that conditions become much moister and the vegetation more mesic at the base of the mountains. This is particularly well seen along the south-eastern edge of the area, where rainstorms originating in the Crowsnest Pass apparently debouch at Elko and follow the mountains south. Elko itself, at the entrance to the Pass, has an annual precipitation of 19.38 in., while as far south as Flagstone the vegetation is of a mesic type, streams are common, and irrigation and mixed farming occur, with even an occasional orchard.

In addition, there is a slight general change in the vegetation, not noticeable to the ordinary observer, in travelling from south to north, due to the varying degree to which certain plants, apparently of southern origin, have been able to establish themselves. Thus the reedgrass (*Calamovilfa longifolia* (Hook.) Scribn.) occurs chiefly just north of the Montana Boundary; *Eriogonum flavum* Nutt., *Orthocarpus tenuifolius* Benth. and *Phlox rigida* Benth. were only observed as far north as Wasa and Skookumchuck, while the antelope-bush (*Purshia*) stops about 10 miles south of Kootenay River Crossing at Canal Flats.

There are two general types of vegetation, namely, forest and climax grassland.

(1) Forest. This at one time has covered most of the area. Whitford and Craig (1918) state that the area was second only to the Coast in the value of its lumbering. Since that time logging has

been carried on so intensively that most of the larger mills have had to close down. It is, therefore, not easy to reconstruct the original picture. The most characteristic tree of the area is the yellow or bull pine (*Pinus ponderosa* Dougl.), which is abundant to Canal Flats, north of which it ceases abruptly. It occupies the hottest, driest areas, where it may form practically pure stands, as at Rampart, Waldo, and parts of Tobacco Plains. More usually it is mixed with Douglas fir (*Pseudotsuga taxifolia* (Lamb.) Britt) in varying degrees, and this may also form almost pure stands. Western larch (*Larix occidentalis* Nutt.) occurs typically in somewhat moister situations, but between Cranbrook and Kimberley as well as elsewhere, all these may be seen growing in the same clump. Spruce, both white and Engelmann's, occurs chiefly along creeks and river-flats, where there is abundance of moisture, but at St. Eugene Mission yellow pine and spruce may be seen growing side by side Rocky Mountain juniper (*Juniperus scopulorum* Sarg) is found occasionally throughout.

The forest type produced by these trees is mostly open (savannah) with a thin ground-cover, chiefly of grass, but with some forbs and dwarf shrubs. Where it has been logged and burned and there is perhaps a little more moisture, the typical dense stands of lodge-pole pine (*Pinus contorta* Dougl.) may occur, as is well seen along the road from Wardner to Jaffray Along creek-bottoms, also, there is often a very dense growth of moisture-loving trees and shrubs—cottonwood (*Populus trichocarpa* T & G), aspen (*Populus tremuloides* Michx.), willows (*Salix sp.*), alder (*Alnus tenuifolia* Nutt.), mountain maple (*Acer glabrum* var. *Douglasii* (Hook) Piper) and red osier dogwood (*Cornus stolonifera* Michx.)

The most characteristic shrub is the antelope-bush (*Purshia tridentata* (Pursh) DC), which occurs throughout except in the moist belt at the foot of the mountains. It reaches its maximum development both as to numbers and size of individuals, on logged-off land southwest of Elko. In the Western Dry Belt this plant extends into British Columbia for perhaps 40 miles north of the 49th Parallel, in the Southern Okanagan and Similkameen, in what is generally regarded as the Upper Sonoran Zone. It is associated there with sage-brush and cactus, both of which, however, extend for some 80 miles farther north into the Kamloops-North-Thompson region. It is not quite clear why *Purshia* should be so abundant in this area, while the more widely-ranging species associated with it in the western region are absent. However, the prevalence here of light sandy and gravelly soils is probably one, and perhaps the most important, reason.

Other common shrubs are roses (*Rosa* sp.), western choke-cherry (*Prunus demissa* Nutt.), mock-orange (*Philadelphus Lewisii* Pursh), bearberry (*Arctostaphylos uva-ursi* (L) Spreng.) and snow-berry (*Symphoricarpos occidentalis* Hook. and less commonly *S. albus* (L) Blake).

(2) Areas of climax grassland are small, the two most important being St. Mary's Prairie, between Cranbrook and Marysville, and Tobacco Plains, adjoining the Montana Boundary. These were noticed by the early explorers and travellers. (Tobacco Plains is said to have received its name from a much-prized strain of tobacco cultivated by the Indians which drew large assemblies from the surrounding country for trade. No trace of tobacco, growing wild or cultivated, could be found by the present writer.) Both St. Mary's Prairie and Tobacco Plains (but especially the latter) have been excessively overgrazed, and it is not easy to infer the nature of the original vegetation. Relatively undisturbed patches are small and not typical in situation of the general area. However, in such places blue bunchgrass (*Agropyron spicatum* var. *inerme* Heller) is usually the dominant grass, and as this is one of the best forage grasses it seems probable that it at one time occurred over the whole area and has disappeared through overgrazing. Rough fescue (*Festuca scabrella* Torr.) is also frequent throughout, as is also Idaho fescue (*F. idahoensis* Elmer), which seems to stand grazing better and is often dominant on St. Mary's Prairie. Other abundant grasses are *Koeleria cristata* (L) Pers., *Poa secunda* Presl and *Stipa columbiana* Macoun. Associated plants are poison camass (*Zigadenus venenosus* Wats.), *Delphinium bicolor* Nutt., *Heuchera glabella* T & G, poison milk-vetch (*Astragalus serotinus* Gray), silky lupin (*Lupinus sericeus* Pursh), *Antennaria parvifolia* Nutt., *Arnica sororia* Greene, dwarf Erigerons of several species, and *Gaillardia aristata* Pursh. It seems, therefore, that the original vegetation here was the *Agropyron-Festuca* association, or Upper Grassland Zone, as described by Tisdale (1947) and Spilsbury and Tisdale (1944). It differs from this, as described from the Western Dry Belt, in the much greater abundance of Idaho fescue and by the presence of a number of species peculiar to the Rocky Mountain region. (*Linanthus septentrionalis* Mason, a species little known in British Columbia was found to be frequent and often abundant on overgrazed range).

While the above applies apparently to most of the area, there are "islands" of considerable size, especially on Tobacco Plains, which are almost pure stands of *Stipa comata* Trin. & Rupr. or *Bromus tectorum* L., or mixed with *Poa secunda*. Tisdale (1947) considers such associations as derived by overgrazing from what was originally

Middle Grassland Zone, so this zone may also have been represented in the hotter drier places (chiefly on gravel).

Where overgrazing has been worst, grass may have almost disappeared and other vegetation taken its place In some cases patches of many square yards are occupied with false dandelion (*Agoseris glauca* (Pursh) Greene), a tap-rooted perennial. An annual woolly composite, *Filago arvensis* L., introduced from Europe, and not known to occur elsewhere in North America, is very abundant on much of the overgrazed range south of Elko, in some places almost taking possession. It appears to be unpalatable to stock.

Skookumchuck Prairie, about 23 miles north of Fort Steele, is a small level area on gravel soil. The grass cover is almost pure *Stipa comata*, with some *Bromus tectorum* and *Koeleria cristata* in places. Forbs are abundant, *Erigerons*, pasture wormwood (*Artemisia frigida* Willd.), *Antennaria parvifolia*, bitter-root (*Lewisia rediviva* Pursh), *Phlox rigida*, *Eriogonum flavum*, woolly plantain (*Plantago Purshii* R & S), etc.

A considerable area of prairie at Wasa, about 12 miles north of Fort Steele, but on the east side of the Kootenay River, seems to be of the same character. Both are apparently derived from Middle Zone Grassland by overgrazing.

Correlation between soil formation and plant cover

Attempts to correlate the plant cover with the underlying soil formation, as determined by the soil surveyors, were not successful Local variations of exposure, drainage and precipitation, apparently outweigh the difference between the soil types. This is well seen in observing the tree cover on the Elko Gravelly Soil Association from the old Newgate Railway Station to the present Flagstone Post Office and northwards. Almost pure stands of yellow pine change to Douglas fir, become mixed with lodge-pole pine and western larch, and along the base of the mountains with aspen, alder, and mountain maple. In other words, the complete extremes of forest cover in the whole southern Trench may occur on the same soil formation. In the case of the grassland the situation is further complicated by overgrazing and secondary associations.

Certain plants, however, are indicative of specially hot, dry situations. *Eriogonum flavum*, a perennial restricted in British Columbia to this section, is one of them. A bed of its bright yellow flower-clusters, often conspicuous at a distance, is an almost certain indication of a gravel soil. The less common *Penstemon eriantherus* Pursh likes a similar habitat, while the lesser paintbrush (*Orthocarpus* *tenuifolius)*, an annual, is very common in such situations, maturing and dying down early.

As elsewhere in the Dry Belt, alkali-grass (*Distichlis stricta* (Torr.) Rydb.) and the poisonous arrow-grass (*Triglochin maritima* L.) were found to be indicative of moist alkali spots; for example around the ponds at Rampart and on the flood-plain of the Kootenay River at Doyle. *Poa juncifolia* Scribn., not uncommon in the area, is supposed to indicate alkali in drier situations.

Transitional areas

On the east the southern section of the Trench ends abruptly against the Rocky Mountains, except at the entrance to the Crowsnest Pass, where Elko is located. Even here, however, the change is abrupt. Within a few hundred yards, travelling east from Elko into the Pass, all the characteristic plants of the Trench have disappeared and been replaced by those of the moister Canadian Zone.

On the west, from Cranbrook, the change is more gradual. *Purshia* does not extend much beyond Cranbrook; the last of the characteristic Rocky Mountain plants, *Hedysarum sulphurescens* Rydb., disappears about 8 miles west of the town. Before reaching Moyie, bracken (*Pteridium aquilinum* var. *pubescens* Underw.) appears, together with bunch-berry (*Cornus canadensis* L.), thimble-berry (*Rubus parviflorus* Nutt.), and other plants of the Canadian Zone, though the yellow pine persists more or less to Kingsgate. Between Yahk and Kitchener the highway lies mostly in the Canadian Zone; western larch, hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and cedar (*Thuja plicata* D. Don) are common; balsam fir (*Abies grandis* Lindl.) is frequent and white pine (*Pinus monticola* Dougl.) occasional. Bracken and thimble-berry are abundant. The cultivated bench lands of the Creston district are in the upper levels of the Arid Transition Zone.

II. MIDDLE SECTION

This extends from Canal Flats northwards nearly to Brisco. The valley floor is occupied by the Columbia and Windermere Lakes, and farther north by extensive swamps and flood-meadows of the sluggish Columbia River. No study was made of this semi-aquatic vegetation, as having an abundance of moisture, it remains fairly constant over large areas, and bears little relation to changes in land conditions.

The benches are mostly rather steep, with comparatively little level land. The vegetation, although still of the Arid Transition type, indicates a less arid condition than obtains in the southern section, but this is probably due less to higher precipitation than to a more retentive soil. (The average precipitation for the six months, April to September, is 6.94 inches at Invermere as against 6.93 at Cranbrook.) Porous gravelly soils and subsoils cover much of the southern area, while in the middle section there is much deep, fine silt, well seen in the "hoodoos" at Dutch Creek.

The most conspicuous difference in the forest cover is the absence of the yellow pine (*P. ponderosa*), which thins out rapidly and ends about 4 miles north of Canal Flats. Douglas fir now becomes the dominant tree, often forming almost pure stands, mostly of an open character, on the lower benches. These have probably been denser in the past, and thinned out by selective logging. Scattered trees of Rocky Mountain juniper (*Juniperus scopulorum*) are frequent in the Douglas fir association.

In moist hollows aspen, cottonwood, and birch (both *Betula fontinalis* Sarg. and *B. papyrifera* var. *occidentalis* (Hook.) Sarg.) occur. Western larch does not seem to descend much below 3500 feet, but above that is often abundant. Along the creeks spruce (both Engelmann's and white), mountain fir (*Abies lasiocarpa* (Hook.) Nutt.), mountain maple and white pine occur.

The shrubs are much the same as farther south except that antelope-bush (*Purshia tridentata*) has disappeared. Dwarf Oregon grape (*Berberis repens* Lindl.), *Rosa* sp., bearberry (*Arctostaphylos uva-ursi*), *Shepherdia canadensis* (L) Nutt., western snowberry (*Symphoricarpos occidentalis*), and rabbit-brush (*Chrysothamnus nauseosus*) are all frequent.

Of herbaceous plants a number already mentioned as peculiar to the southern section have disappeared, but most of the others persist. A few years ago the dry southern slopes of hillside ranges at Windermere were brilliant with scarlet mallow (*Sphaeralcea coccinea* (Nutt.) Rydb. known otherwise in British Columbia only in the Kamloops district, but this has disappeared, at least temporarily, probably as a result of overgrazing with sheep.

It is doubtful if there has been any extent of original grassland in this section. Probably most of the present range-land was open forest, cleared or thinned-out by logging. Most of the ranges have been so overgrazed that it is difficult to infer the original composition. However, from examination of some range at Fairmont which had been allowed to recover, it would appear that typical *Agropyron spicatum* (Pursh) Scribn. & Sm. replaces var. *inerme*, the dominant form in the southern section.

There are three other interesting botanical features of the section:

(1) Two ferns, rare elsewhere in British Columbia, are abundant in the dry limestone cliffs above Fairmont, a rather unusual habitat for ferns. These are *Cheilanthes Feei* Moore and *Pellaea Suksdorfiana* Butters. Ferns are scarce in both the southern and middle sections, one site of *Cystopteris fragilis* (L) Bernh. and a few collections of *Woodsia scopulina* D. C. Eaton and *W. oregana* D. C. Eaton being the only ones seen in addition to the one to be mentioned later.

(2) A number of plants found in Alberta, and mostly with an extensive range east of the Rocky Mountains, but not otherwise known from British Columbia west of the Rockies. These are *Andropogon scoparius* Michx. and *Gaura coccinea* Pursh; *Arabidopsis glaucum* (Nutt.) Rydb. and *Plantago eriopoda* Torr.; *Calamagrostis montanensis* Scribn.

The first two are known only from small solitary sites at Fairmont and Windermere Beach respectively, both popular tourist resorts, and may perhaps have been accidentally introduced by man; the next two occur over a larger area of alkali meadow at Windermere; the last-named extends for some miles north and south of where the highway from Banff, Alberta, enters the Trench and extends across the valley to the benches of Invermere and Wilmer. In a small protected area above Windermere Beach it proved to be the dominant grass. There are two specimens in the United States National Herbarium collected by J. Macoun in 1904 in "Rocky Mt. Park," which may be the present Kootenay or Banff National Park, and the species appears to be common in Alberta. It seems possible, therefore, that it may have migrated along the passes through which the Banff-Windermere highway now goes, perhaps helped inadvertently by human agency.

(3) The flora of the hot springs at Fairmont. Here the most remarkable plant is the maiden-hair fern of the florists (*Adiantum capillus-Veneris* L.). A few years ago this was extremely abundant not only around the spring from which water was carried to the swimming-pool, but sprouted out in hundreds from the chinks of the wooden flume. "Improvements," including a new flume, have destroyed much of it, but there is still a fine bed of it around an "undeveloped" spring. As the nearest stations as given in Abrams (1923) are Utah and southern California, one's first surmise is that it must have been planted by some enthusiast. Apart, however, from the fact that there is no knowledge or tradition of this among the local residents, there is some historical evidence that it was well-established at the earliest stage of the settlement of the valley. (A fuller discussion appears in Eastham, 1949.)

Other interesting and unusual plants found around the hot springs or along their effluents are *Triglochin palustris* L., *Muhlenbergia glomerata* (Willd.) Trin., *Panicum thermale* Boland (abundant, and the only known station in B.C.), *Juncus Torreyi* Cov., *Epipactis gigantea* Dougl., and a white-bracted *Castilleja* of uncertain taxonomic status—possibly an undescribed species.

III. NORTHERN SECTION

This section does not appear to possess any features of special interest, but, for various reasons, was studied more cursorily than the other two. The change to the Canadian Zone begins gradually some miles south of Brisco, which itself is 49 miles south of Golden. The Mariposa lily (*Calochortus macrocarpus* Dougl.), a common plant from the Montana Boundary, was last seen at a point 55 miles south of Golden. *Chrysothamnus* ceased a few miles earlier. The Douglas fir begins to show a denser growth and to form thicker stands. The grass-cover becomes taller and thicker, and Kentucky blue-grass (*Poa pratensis* L.) becomes the dominant grass along the roadsides, replacing the *Koeleria*, *Stipas* and *Agropyron* bunch-grasses. Aspen, birch, and spruce become steadily more plentiful, but cedar (*Thuja plicata*) was not noticed until near Golden.

REFERENCES

ABRAMS, L. (1923). Illustrated Flora of the Pacific States, 1: 24.

EASTHAM, J. W. (1949). *Adiantum capillus-Veneris* L. in British Columbia. Can. Field-Nat., **63**: 112-114.

SPILSBURY, R. H. and TISDALE, E. W. (1944). Soil-plant relationships and vertical zonation in the southern interior of British Columbia. Sci. Agric., **24**: 395-436.

TISDALE, E. W. (1947). The grassland of the southern interior of British Columbia. Ecol., **28**: 346-82.

WHITFORD, H. N. and CRAIG, R. D. (1918). Forests of British Columbia. Ottawa.

MÉMOIRES DE LA SOCIÉTÉ ROYALE DU CANADA
TOME XLII : TROISIÈME SÉRIE : JUIN 1949
SECTION CINQ

L'Action bactériostatique du glycocolle et les besoins du rat en pyridoxine

Par EDOUARD PAGÉ et ROSAIRE GINGRAS

Présenté par GEORGES MAHEUX, M.S.R.C.

LES propriétés toxiques du glycocolle ont fait l'objet de plusieurs études. En 1944, Hier et ses collaborateurs (1944) démontraient que l'addition de glycocolle au taux de 6 pour cent de la ration du rat provoquait un retard de la croissance. Martin (1946) constatait que le glycocolle est plus toxique lorsque la ration est dépourvue de riboflavine que lorsqu'elle est complète. Il est également établi que l'emploi de certaines protéines à faible teneur en tryptophane entraîne une carence en acide nicotinique, carence rémédiable par l'administration de tryptophane ou d'acide nicotinique (Krehl, Sarma *et al.*, 1946). On observe les mêmes résultats si l'on ajoute du glycocolle à des régimes pauvres en tryptophane, à condition que les glucides alimentaires soient constitués de sucrose et non de dextrine (Krehl, Henderson *et al.*, 1946). Le glycocolle augmenterait donc les besoins du rat en acide nicotinique. Enfin, Snell et Guirard (1943) ont rapporté que l'effet toxique du glycocolle sur la croissance de Streptococcus lactis pouvait être partiellement corrigé par un surcroît de pyridoxine dans le milieu de culture. Pour leur part, Pagé et Gingras (1946) ont observé que l'administration de glycocolle par gavage est sans effet chez le rat recevant une ration complète, mais qu'elle provoque rapidement l'arrêt de la croissance chez le rat recevant une ration dépourvue de pyridoxine. Ils en ont conclu que le glycocolle augmente les besoins du rat en pyridoxine.

Les résultats que nous rapportons ici confirment le fait que le glycocolle augmente les besoins *exogènes* du rat en pyridoxine. Certaines observations additionnelles portent à croire cependant qu'il s'agit d'une simple action *bactériostatique*, les besoins exogènes étant augmentés par suite de la diminution des synthèses intestinales, synthèses qui normalement contribuent leur part de pyridoxine au métabolisme. Un résumé de ces travaux a déjà été publié (Pagé et Gingras, 1947).

PROTOCOLE EXPÉRIMENTAL ET RÉSULTATS

Le but des ces expériences était de déterminer si l'addition de quantités substantielles de glycocolle à la ration du rat augmente les besoins en pyridoxine. A cette fin, nous avons d'abord présumé qu'un taux de pyridoxine alimentaire de 0.2 mg. par 100 grammes de ration est normalement suffisant, même si cette ration contient jusqu'à 30 pour cent de caséine, ce qui représente une quantité d'azote métabolisable dépassant celle des rations expérimentales contenant du glycocolle. Nous avons de plus supposé qu'il y avait augmentation des besoins en pyridoxine si ce taux devenait insuffisant à la suite de l'addition de glycocolle au régime.

Besoins courants du rat en pyridoxine

Nous nous sommes d'abord assurés que le taux de base choisi (0.2 mg. pour cent de pyridoxine) était satisfaisant. Les rations contenaient 30 pour cent de protéine, 2 pour cent de cellulose (Cellu flour), 1 pour cent d'huile Mazola, 4 pour cent de sels minéraux, et 60 pour cent de sucrose. Les rats recevaient en plus, chaque semaine, un supplément oral de vitamines A, D, et E. Les sels minéraux étaient constitués du mélange U.S.P. XII, No. 2, auquel nous avions ajouté les sels suivants : $CuSO_4.5H_2O$, 0.3 ; KI, 0.7 ; $MnSO_4.4H_2O$, 5.0 ; et $ZnCl_2$, 0.2 grammes, par kilogramme du mélange. La teneur en vitamines était la suivante : (par 100 grammes de ration) thiamine, 0.4 ; riboflavine, 0.1 ; pyridoxine, 0.2 ou 2.0 ; panthothénate de calcium, 3.0 ; acide nicotinique, 3.0 ; 2-méthyl-1,4-naphtoquinone, 0.1 ; inosite, 10.0 ; et chlorure de choline, 150 mg.

Une première expérience, dont les résultats sont inscrits au Tableau I (Expérience 1), portait sur deux groupes de 10 rats mâles d'un poids initial moyen de 87 grammes, un groupe recevant 2.0 et l'autre 0.2 mg. de pyridoxine par 100 grammes de ration. Trois semaines plus tard, les deux groupes enregistraient un gain de poids moyen identique, soit 38 grammes par semaine. *Il est donc évident qu'un taux de pyridoxine de 0.20 milligramme par 100 grammes de ration est normalement suffisant.* Dans une seconde expérience, quatre groupes de 10 rats chacun furent utilisés, deux groupes recevant 30 pour cent de caséine dans la ration et les deux autres, 20 pour cent de caséine et 10 pour cent de gélatine. Les mêmes deux niveaux de pyridoxine que précédemment furent comparés dans chaque cas. L'effet possible de la gélatine fut étudié parce que Hier et ses collaborateurs (1944) lui avaient trouvé un effet toxique sur la croissance dans certaines conditions. Il est clair (Tableau I, Expérience 2) que de nouveau, *un apport supplémentaire de pyridoxine s'avère superflu.*

TABLEAU I

TAUX DE CROISSANCE DE RATS À DIVERS NIVEAUX DE PYRIDOXINE

Expérience no.	*Groupe no.*	*Nombre de rats par groupes (mâles)*	*Poids initial moyen (grammes)*	*Pourcentage de caséine*	*Pourcentage de gélatine*	*Pyridoxine-HCl Mg. %*	*Gains moyens par semaine (grammes) Nombre de semaines*	
							3	4
1	I	10	87	30	0	2 0	38	
	II	10	87	30	0	0.2	38	
2	I	10	71	30	0	2.0	37	36
	II	10	72	30	0	0.2	35	33
	III	10	72	20	10	2.0	36	34
	IV	10	72	20	10	0.2	36	35

La différence entre les groupes I et II n'est pas significative («p» dépasse 0.20).

Il s'agissait maintenant de vérifier si un tel apport additionnel de pyridoxine exercerait un effet favorable chez le rat recevant du glycocolle dans son régime alimentaire.

Effets du glycocolle sur les besoins du rat en pyridoxine

Les rations de base utilisées contenaient comme les précédentes, 4 parties pour 100 d'huile, 4 de sels minéraux et 2 de cellulose. Les quantités de caséine et de glycocolle sont indiquées au Tableau II, la balance de la ration étant constituée de sucrose. Les vitamines furent incorporées dans la ration au même taux qu'indiqué précédemment, pour les expériences 5 et 6. Au cours des expériences 3 et 4, la teneur en vitamines était la suivante : thiamine, 0.5 ; riboflavine, 0.5 ; pyridoxine, 0.2 ou 1.0 ; pantothénate de calcium, 5.0 ; acide nicotinique, 5.0 ; 2-méthyl-1, 4-naphtoquinone, 0.1 ; inosite, 10.0 ; et chlorure de choline, 200 mg. par 100 grammes de ration. Ces variations de teneur en vitamines sont sans portée pratique et furent effectuées uniquement pour fins de standardisation avec d'autres travaux.

En premier lieu, 7 grammes de glycocolle furent ajoutés à 100 grammes du régime de base déjà préparé, ce qui eut pour effet d'abaisser légèrement le taux de caséine de la ration et de réduire le taux de glycocolle, exprimé en pourcentage, à 6.5 pour cent (Tableau II, Expérience 3). Trois groupes de rats furent constitués, le premier étant le groupe témoin (ration de base sans glycocolle), le second recevant 6.5 pour cent de glycocolle et 1.0 mg. pour cent de pyridoxine, et le troisième, la même quantité de glycocolle mais seulement 0.2 mg. pour cent de pyridoxine. Au bout de trois semaines, les rats témoins (groupe I) accusaient des gains de poids beaucoup plus élevés que les autres mais il existait néanmoins une différence statistiquement significative entre les deux groupes au glycocolle, à l'avantage de celui recevant le niveau supérieur de pyridoxine (groupe II). Au cours des deux semaines suivantes, les rats du groupe III reçurent quotidiennement par voie parentérale 100 microgrammes de pyridoxine. Leurs gains de poids par semaine pour cette période s'établissaient à 14 grammes, comparativement à 11 grammes pour le groupe II et 24 grammes pour le groupe I.

Les mêmes rations furent utilisées une seconde fois avec cette différence que les rats furent d'abord soumis durant 5 semaines à un régime dépourvu de pyridoxine afin d'épuiser tout au moins en partie leurs réserves en cette vitamine. Les résultats furent semblables aux précédents, l'apport additionnel de pyridoxine favorisant

TABLEAU II

TAUX DE CROISSANCE DE RATS RECEVANT DU GLYCOCOLLE ET DIVERS APPORTS DE PYRIDOXINE

Expérience no.	*Groupe no.*	*Nombre de rats par groupe*		*Poids initial moyen (grammes)*	*Pourcentage de caséine*	*Pourcentage de glycocolle*	*Pyridoxine—(See Table I) HCl mg. %*	*Gains moyens par semaine (grammes)*	*Valeur de «p»*
		mâles	*femelles*						
3	I	3	6	49	18.0	0	1.0	28	0.01
	II	3	6	49	16.8	6.5	1.0	20	
	III	3	6	50	16.8	6.5	0.2	16	0.01
4	I	6	6	113	18.0	0	1.0	26	0.10
	II	6	6	115	18.0	0	0.2	24	0.02
	III	6	6	113	16.8	6.5	1.0	20	0.01
	IV	6	6	113	16.8	6.5	0.2	16	
5	I	4	5	46	26.3	0	0.2	23	
	II	4	5	47	20.0	5.0	2.0	23	0.20
	III	4	5	47	20.0	5.0	0.2	21	
6	I	4	7	65	20.0	6.5	2.0	23	0.01
	II	4	7	64	20.0	6.5	0.2	19	

Durée de toutes les expériences: trois semaines.

Seule une valeur de «p» (probabilité) inférieure à 0.05 indique une différence significative.

légèrement une croissance plus rapide. Il est aussi à noter, qu'il n'y a pas de différence statistiquement significative entre les deux groupes témoins (Tableau II, Expérience 4).

Au cours d'une expérience subséquente, le taux de caséine fut porté à 20 pour cent et celui du glycocolle fut réduit à 5 pour cent pour les rats recevant cet acide aminé. La ration des rats témoins contenait 26.3 pour cent de caséine, soit une quantité d'azote équivalente à celle de l'autre ration. Dans de telles conditions, le glycocolle n'eut pas d'effet déprimant sur la croissance, et les deux taux de pyridoxine aboutirent à des résultats identiques (Tableau II, Expérience 5).

Enfin, le taux de glycocolle fut rétabli à 6.5 pour cent, la ration contenant par ailleurs 20 pour cent de caséine. Au bout de trois semaines, le groupe I (2.0 mg. pour cent de pyridoxine) accusa un gain de poids peu supérieur à celui du groupe II (0.2 mg.) mais cette différence était nettement significative (Tableau II, Expérience 6). Beaucoup plus tard, tous les rats maigrirent à peu près également, et il devint évident qu'une insuffisance en pyridoxine n'était en fait qu'un à-côté du problème de la toxicité du glycocolle.

Effets du glycocolle sur les besoins du rat en biotine

Soixante-quinze jours après le début de l'expérience précédemment décrite, dix des rats furent alors répartis en deux groupes (4 femelles et 1 mâle) de comportement antérieur à peu près semblable. On fixa alors le taux de pyridoxine à 0.5 mg. par 100 grammes de ration. Les rats d'un groupe reçurent chaque jour, par voie sous-cutanée, 20 microgrammes de biotine tandis que ceux de l'autre reçurent un supplément de 1 pour cent d'un concentré de foie (Wilson's Liver

TABLEAU III

COMPOSITION DES RATIONS

(grammes par 100 grammes du mélange)

	GROUPE I	GROUPE II	GROUPE III
Caséine	18	18	18
Glycocolle	0	10	10
Foie	0	0	1
Huile	4	4	4
Sels minéraux	4	4	4
Cellulose	2	2	2
Cérélose (glucose)	72	62	61

1-20). Dans les deux cas, la reprise de la croissance fut immédiate (Fig. 1) et les symptômes notés plus haut disparurent. Au bout de deux semaines, on donna, en plus de la biotine, 200 microgrammes d'acide folique. Ce supplément additionnel ne semble pas avoir eu d'effet particulier sur le comportement des rats. L'action de la biotine est bien nette et porte à croire que ces rats étaient véritablement carencés en cette vitamine. Au cours d'une expérience subséquente que nous ne décrirons pas en détail, il nous fut impossible de répéter ces résultats mais par contre, l'administration d'acide folique s'avéra efficace.

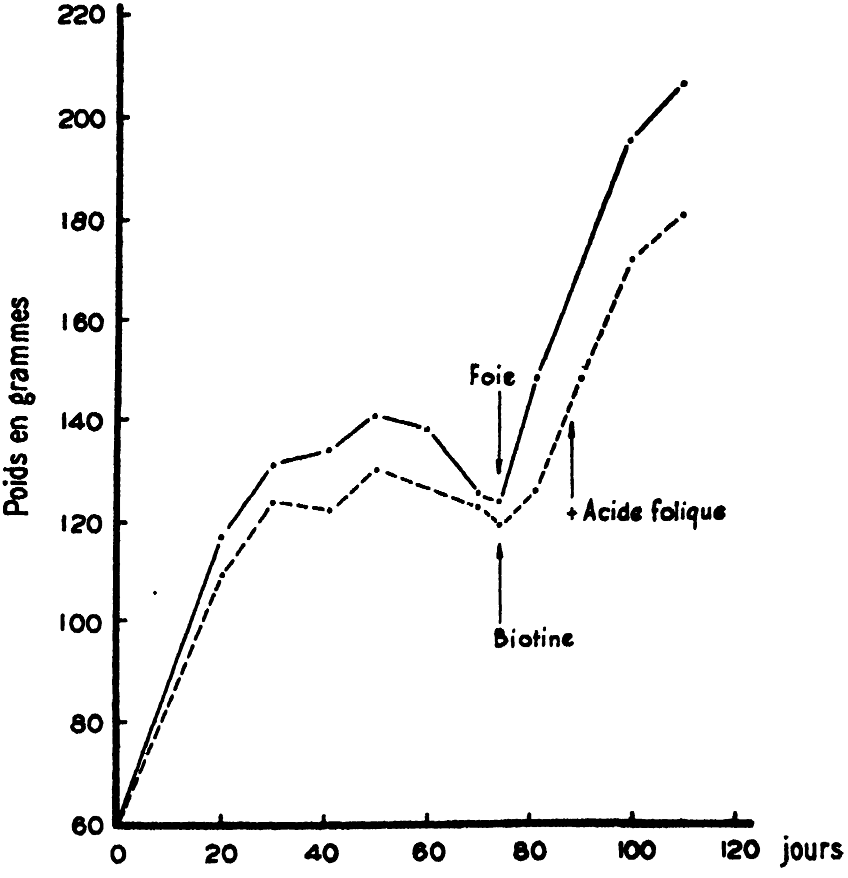


FIGURE I

Trois groupes de 10 rats furent finalement soumis, respectivement, aux régimes décrits au Tableau III. Pour des raisons d'ordre pratique, on remplaça le sucrose par du cérélose.

L'addition d'un concentré de foie eut pour effet de prévenir en grande partie l'action toxique du glycocolle. Quant aux rats intoxiqués, l'administration d'acide folique (200 microgrammes par jour) provoqua chez eux une reprise immédiate de la croissance (Fig. 2).

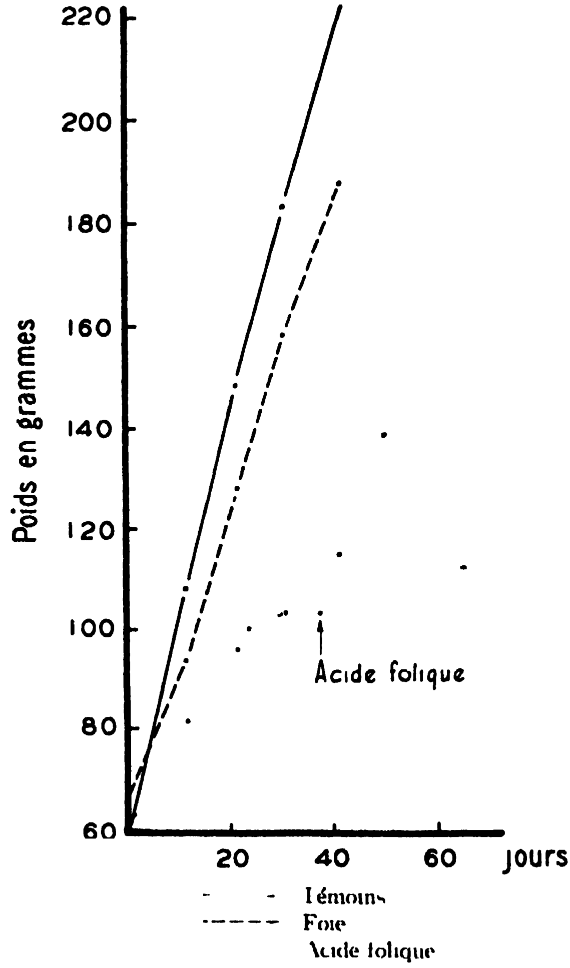


FIGURE II

DISCUSSION

Ces expériences indiquent clairement que l'addition de glycocolle au régime est susceptible de provoquer une avitaminose complexe. Il est peu probable, cependant, que le métabolisme du glycocolle entraîne une *dépense* plus considérable de toutes les vitamines dont le rat a un plus grand besoin *exogène* à la suite de l'incorporation de cet acide aminé dans la ration. En effet, si nous ajoutons à nos observations celles des auteurs déjà cités, le glycocolle augmenterait les besoins du rat en riboflavine, en acide nicotinique, en pyridoxine, en biotine et en acide folique. Il est plus plausible de supposer que le glycocolle exerce une action bactériostatique analogue à celle des sulfamidés. Les ressemblances sont évidentes dans le cas de la biotine et de l'acide folique. Quant à la pyridoxine, Sarma *et al.* (1946) ont démontré que sa synthèse intestinale variait en intensité chez le rat suivant la nature du régime. Utilisant une ration qui ne favorisait pas cette synthèse (sans non plus l'éliminer), ces auteurs ont constaté que le taux minimum de pyridoxine *alimentaire* nécessaire à une croissance optima s'établissait aux environs de 0.15 mg. par 100 grammes de ration. Le niveau de base que nous avons utilisé était de 0.20 mg. et il n'est pas improbable que les besoins *exogènes* du rat en cette vitamine atteignent ou dépassent ce dernier taux si la synthèse de pyridoxine est réduite suffisamment sous l'action d'un agent bactériostatique. S'il en est ainsi du glycocolle, les besoins *endogènes* du rat en pyridoxine ne dépassent guère 0.20 mg. par 100 grammes de ration, puisqu'un taux plus élevé n'a amélioré que très légèrement la croissance de ces animaux. Il faut admettre, toutefois, que l'effet d'un supplément de pyridoxine aurait peut-être été plus marqué en l'absence d'avitaminoses autres.

CONCLUSIONS

L'addition de glycocolle à des rations purifiées augmente légèrement les besoins exogènes du rat en pyridoxine. Le glycocolle provoque également une avitaminose complexe remédiable par la biotine ou l'acide folique. Il semble que l'effet du glycocolle sur les besoins du rat en pyridoxine alimentaire se limite à une diminution de la synthèse intestinale de cette vitamine.

RÉSUMÉ

L'addition de glycocolle à des rations purifiées augmente légèrement les besoins du rat en pyridoxine d'origine *exogène*. Ceci peut être dû à une simple diminution de la synthèse intestinale de cette vitamine. En effet, l'administration prolongée de glycocolle entraîne une carence alimentaire complexe remédiable parfois par la biotine et parfois par l'acide folique.

SUMMARY

The incorporation of glycine into purified rations increases slightly the *dietary* pyridoxine requirements of the rat. However, this may be due simply to a reduced intestinal synthesis of this vitamin since prolonged administration of glycine results in a complex avitaminosis which responds in some cases to biotin, and in others, to folic acid.

REMERCIEMENTS

Les auteurs désirent exprimer leurs remerciements au Conseil National des Recherches du Canada pour son aide matérielle. Ils remercient également les maisons Merck et Lederle qui leur ont fourni, respectivement, une partie de la biotine et de l'acide folique utilisés au cours de ces expériences.

RÉFÉRENCES

HIER, S. W., GRAHAM, C. E. et KLEIN, D. (1941). Proc. Soc. Exp. Biol. Med., **56** : 187

KREHL, W. A., HENDERSON, L. M., de la HUERGA, J et ELVEHJEM, C A. (1946). J. Biol. Chem., **166** : 531.

——— SARMA, P. S., TEPLY, L. J. et ELVEHJEM, C. A. (1946). J. Nutr., **31** : 85.

MARTIN, GUSTAV J. (1946). Proc. Soc. Exp. Biol. Med., **65** : 528.

PAGÉ, E. et GINGRAS, R. (1946). Trans. Roy. Soc. Can., **40**, sec. V : 119.

——— (1947). Rev. Can. de Biol., **6** : 373.

SARMA, P. S., SNELL, ESMOND E. et ELVEHJEM, C. A. (1946). J. Biol. Chem., **165** : 55.

SNELL, E. E. et GUIRARD, B. M. (1943). Proc. Nat. Acad. Sci., **29** : 66.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIII : SERIES III : MAY, 1949
SECTION FIVE

An Experimental Anatomical Study of the Feeling of Pressure

By I. MACLAREN THOMPSON, F.R.S.C., L. W. DENNY,
H. S. KIMBALL, and J. C. LUCE[1]

THE primary cutaneous sensations are usually listed as touch, pressure, pain, heat, and cold; to each are commonly attributed its own specific end-organs and nervous pathways to the sensorium. But pressure has presented difficulties.

Because electrical stimulation of nerves supplying skin in conscious people aroused no feeling of pressure, Heinbecker, Bishop, and O'Leary (1934) opined that cutaneous nerves contain no fibres mediating the sense of pressure. But the striking experimental elevation of pressural thresholds upon stimulating such nerves with suitable alternating currents, first reported by Thompson (1933), and in detail by Thompson *et al.* (1934) in 142 experiments upon the radial nerve, led us to maintain that fibres somehow concerned with the sense of pressure do exist in cutaneous nerves. This view receives further support from 122 additional experiments involving seven nerves[2] reported in the present communication.

CONCERNING THE NATURE OF THE FEELING OF PRESSURE

Bazett (1934) has suggested the following explanatory reconciliation of the results of Heinbecker, Bishop, and O'Leary with those of Thompson, Banks, Barron, Fratis, and Mattison:

> . . . The pressure sense is not so much a sensation as a concept, and the conclusion by Heinbecker, Bishop, and O'Leary (1934), that pressure is not mediated by cutaneous nerves because electrical stimulation of such nerves gives no sensation

---

[1] From the Departments of Anatomy of the Universities of California and Manitoba. This investigation was aided by grants to the senior author from the Board of Research of the University of California, and from the University of California chapter of the Society of Sigma Xi, to whom appreciative acknowledgments are tendered. The procaine used was kindly donated by Ely Lilly and Company. The nerve-blocking injections were skilfully performed by Dr. A. A. Koneff.

[2] Counting the dorsal branch of the ulnar as a separate nerve.

of pressure, is not warranted. Electrical stimulation could not be expected to reproduce the pattern of a concept. On the other hand, Thompson and his co-workers find that electrical stimulation of cutaneous nerve trunks of the hand masks pressure sensations even more than those of touch, but has little effect on other sensations. If pressure sensations depend on spatial summation of impulses of low frequency from many receptors, their special sensitivity to this masking effect is not surprising.

We now present the results of experiments bearing upon Bazett's suggestion, and some discussion thereof.

Thompson (1935b, 1943) has adduced experimental evidence that the elevation of sensory thresholds by stimulating cutaneous nerve trunks is a *central* phenomenon termed masking. Thompson and Kimball (1938) have applied the term "reversed masking" to elevation of the liminal alternating current for eliciting a fluttering sensation by percutaneous stimulation of a nerve, such elevation being produced by stimulating end-organs in the area supplied by the nerve. The experiments mentioned by Thompson and Kimball, together with others, are here reported and discussed in detail.

In these experiments, each involving three nerves and four human subjects, the cutaneous area supplied by the nerve stimulated electrically was subjected to continuous *tactual* stimulation by holding it lightly against the rim of a slowly revolving wheel made of soft cloth, resembling the buffer-wheel used in some trades. The wheel was driven by an electric motor at a rate just sufficient to evoke a sensation of touch, but not fast enough to generate heat by friction with the skin. The hand was placed so that the wheel touched the nerve area selected, radial, median or ulnar.

Four types of tactual experiment were performed. (1) *Ipsilateral touch on same area:* touch was applied to the cutaneous area supplied by the nerve stimulated. (2) *Ipsilateral touch on different area:* touch was applied to a cutaneous area other than that supplied by the nerve stimulated, but on the same hand. (3) *Contralateral touch on same area:* touch was applied to the area supplied by the same nerve as that stimulated, but on the opposite hand; e.g., left radial area touched, right radial nerve stimulated. (4) *Contralateral touch on different area:* touch was applied to a cutaneous area other than that supplied by the nerve stimulated, and on the opposite hand; e.g., right median area touched, left ulnar nerve stimulated.

In each experiment three successive flutter thresholds were observed under normal conditions, the limb resting comfortably on the bench with the selected area of the hand in light contact with the motionless wheel. The motor was then started, arousing the sensation of touch in the desired area of skin, and three successive flutter thresholds were again observed. Each threshold is expressed as the product of the milliampères and volts of the minimal current arousing the fluttering sensation; the use of this approximation to milliwatts is discussed by Thompson *et al.* (1934). For each experiment the mean of the three normal thresholds (expressed as *ma.v.*) was subtracted *algebraically* from the mean of the three experimental thresholds; if a stronger current was required to evoke flutter during tactual stimulation, the difference was positive; if a weaker current was needed, the difference was negative.

To study the effect of the feeling of *pressure* on the flutter threshold, pressure was applied to the hand, and an ipsilateral or a contralateral nerve supplying the hand was stimulated and the thresholds recorded as just described; the two types of experiment are designated *ipsilateral pressure* and *contralateral pressure.* Pressure was applied by wrapping the arm-band of a Riva-Rocci sphygmomanometer round the hand and inflating it to a pressure of about 40 mm. Hg. This caused a continuous feeling of pressure over most of the hand, lasting throughout the experiment. The circulation was not disturbed, as judged by the colour of the nails. In each experiment, two to four flutter threshold values were determined after the cuff had been wrapped round the hand, but before inflation. Pressure was then applied by inflating the cuff, and after two minutes the flutter threshold was observed. The mean of the normal thresholds was subtracted algebraically from the threshold after two minutes of pressure. To explain the reason for the slight difference in taking the experimental thresholds during pressural and during tactual stimulation would occupy excessive space. These pressural experiments involved the same three nerves and four subjects as those on touch.

The results of these experiments are summarized in Table I.

The significance of each mean in column 3 was subjected to the *t*-test, the results appearing in column 4. It is clear that ipsilateral touch on the same area has a reversed masking effect unapproached by any other variety of sensory stimulation tested. The reversed masking effect of ipsilateral touch on a different area is interesting. Though less than one-fifth as effective as touch on the same area, it is nevertheless statistically significant; this we take as an expression of the remarkable power of touch to effect reversed masking, even more widely than might be expected. Contralateral touch, and pressure on either side, failed completely to produce reversed masking. Indeed, a small lowering of flutter threshold occurred. But this lowering occurs under such a variety of circumstances studied by

TABLE I

(1) *Peripheral stimulus*		(2) *Number of experiments*	(3) *Mean rise in nerve-stimulation threshold* (ma. v)	(4) P_t
(A) Ipsilateral touch	same area	20	+ 3.44	$0.01 > P$
	different area	19	+ 0.60	$0.01 > P$
(B) Contralateral touch	same area	26	− 0.13	$0.2 > P > 0.1$
	different area	24	− 0.13	$0.1 > P > 0.05$
(C) Pressure	ipsilateral	18	− 0.26	$0.01 > P$
	contralateral	18	− 0.34	$0.01 > P$

Kimball, but not reported here, that we are convinced that it does not depend upon the peripheral tactual and pressural stimulation in these experiments.

What interests us is the failure of ipsilateral pressure to produce reversed masking whereas touch is so powerful to do so. This may be explained in terms of the rapid adaptation of certain end-organs to tactual stimulation (Adrian, 1928), leaving their fibres available for electrical stimulation, and their sensorial connections free to perceive the resulting sensation. In other words, the nerve fibres first stimulated by a current gradually increasing from zero are tactual (as is shown by the reversed masking effect of peripheral touch), but they are not *essential* to the awareness of pressure.

Let us attempt to re-interpret these and other experimental results along the lines of Bazett's suggestion that pressure is not a primary sensation but a mental concept. Electrical stimulation of a nerve (Heinbecker, Bishop, and O'Leary, 1934) or of the cerebral cortex (Penfield and Boldrey, 1937) fails to evoke a feeling of pressure because it does not reproduce the requisite spatial pattern of sensory impulses. The susceptibility of pressure to direct masking (especially in the hand—*vide infra*) bespeaks disorganization of that pattern by electrical stimulation of a nerve. In our experimental blocking of nerves with procaine (Thompson, 1935b, 1943), awareness of pressure disappeared before that of touch. Now procaine affects nerve fibres (even of a single type) not simultaneously but progressively as an adequate concentration penetrates the nerve trunk; hence the *pattern* of sensory impulses is disorganized before all the impulses are blocked; at that temporary stage fibres still active individually may arouse the sensation of touch although the combinations necessary to develop the concept of pressure are no longer possible. The failure of pressure to effect reversed masking simply means that the endings and fibres concerned with light touch are not an *essential* part of the combination leading to the concept of pressure; this explanation is supported by our experiments on deep pressure through anaesthetized skin (*vide infra*). It may be remarked parenthetically that not all afferent nerve impulses generated by pressure produce a feeling of pressure; this is notably exemplified by the pressure receptors in the carotid sinus, and probably by the Pacinian corpuscles in the mesentery (Sheehan, 1933).

COMPARATIVE MASKING OF PRESSURE AND OF TOUCH

Thompson *et al.* (1934) observed that when, in conscious people, they stimulated the radial nerve through the skin by appropriate alternating currents, differential elevation of sensory thresholds occurred in the peripheral area supplied by the nerve, the threshold for pressure being raised higher than that for touch in all their experiments but one; they realized that this relationship between the experimentally elevated thresholds for pressure and for touch might be a peculiarity of the nerve with which they worked, or of the region supplied by it.

To test this, we have examined the results of 146 experiments wherein the thresholds for pressure and for touch were elevated by this means: these comprise the 24 "combined runs" of Thompson *et al.* (1934) and 3 additional "combined runs," together with 119 further experiments on pressure and touch only. From the records of the "combined" experiments we have extracted the threshold rises of pressure and of touch at the end of each experiment. In each of the new experiments on pressure and touch only, a single strength of current was introduced, sufficient to evoke a fairly strong throbbing or tingling sensation, and the rise in threshold above the immediately preceding normal value at the same point on the skin was ascertained. For purposes of comparison, each threshold rise is expressed as percentage of the immediately preceding normal threshold. We are concerned with the *difference* between the *percentage* rise in the threshold for pressure and that for touch produced at the same place by the same current: of this difference we have 146 values. For uniformity and clarity, we express the difference in the form *pressure—touch*: when the percentage elevation of pressure was the greater, the difference is stated to be positive; when touch was raised more than pressure, the difference is stated as negative. Although the frequencies of the alternating currents whereby the nerves were stimulated have no evident bearing upon the results herein considered (*vide* Thompson *et al.*, 1934), it may be mentioned that the frequencies of the "combined" experiments ranged from 53 to 999 cycles per second; those of the experiments on pressure and touch only ranged from 106 to 400 cycles per second, most of the experiments being performed at 213 cycles per second. Of course a constant frequency was maintained throughout each experiment. Actually, 124 experiments on pressure and touch were performed, but the results of five were omitted from the computations, because in each of these the rise in one of the thresholds (pressure or touch) so exceeded not only our general experience, but also the rest of our experience with that nerve in that person, that we unhesitatingly ascribe it to a technical blunder. An analytical summary of the results is presented in Table II.

In column 4, a + before the figure indicates that in that set of experiments the average pressural threshold was elevated more than that for touch, whilst a — before the figure means that the average tactual threshold was raised more than that for pressure. Column 5 indicates the significance of each mean value in column 4, as estimated by the *t*-test. Our tabulated P's are half the values of those extracted from the *t*-table, for we are assessing the significance of each mean in respect of algebraic sign as well as of magnitude. The "aberrant" values in column 6 are those of opposite algebraic sign to that predominating in the particular set of experiments, as indicated by the sign prefixed to the mean; for example, in the experiments on the radial nerve, 36 of the differences were positive (pressure raised more than touch), and 2 were negative (touch raised more than pressure)—i.e., "aberrant." Comparison of the magnitude of each percentage of aberrant values with that of its standard error indicates the reasonableness or otherwise of attributing the aberrations to chance. For example: the 2 aberrant values (5 per cent) in the 38 experiments on the radial nerve may well be deemed fortuitous, and ignored; whereas the 11 aberrant values (39 per cent) in the 28 experiments on the medial cutaneous nerve must be regarded as bespeaking a degree of heterogeneity in the observations beyond that reasonably attributable to chance.

The 38 experiments on the *radial nerve* comprised (*a*) the 24 "combined" experiments of Thompson *et al.* (1934), and (*b*) 14

TABLE II

(1) *Nerve*	(2) *Number of subjects*	(3) *Number of observations*	(4) *Mean value of difference: pressure-touch*	(5) P_t	(6) *Percentage of "aberrant" values, with its standard error*
Radial	7	38	+ 3289	$0.005 > P$	5 ± 11
Median	3	8	+ 4521	$0.005 > P$	12 ± 11
Ulnar (palm)	3	10	+ 1534	$0.005 > P$	0
Dorsal cutaneous	3	15	− 2864	$0.005 > P$	13 ± 9
Ulnar (dorsal branch)	1	10	− 2080	$0.10 > P > 0.05$	50 ± 16
	3	15	+ 1657	$0.005 > P$	0
	Total: 4	25	+ 162	$0.40 > P > 0.35$	20 ± 8
Lateral cutaneous	1	6	+ 1003	$0.25 > P > 0.20$	33 ± 19
	3	16	− 1855	$0.010 > P > 0.005$	12 ± 8
	Total: 4	22	− 1076	$0.10 > P > 0.05$	27 ± 9
Medial cutaneous	1	12	− 324	$0.30 > P > 0.25$	42 ± 8
	2	16	+ 910	$0.10 > P > 0.05$	25 ± 11
	Total: 3	28	+ 398	$0.20 > P > 0.15$	39 ± 9
Total	7	146	—	—	—

additional experiments on this nerve; the conclusion of that group, that for this nerve pressure is much more susceptible to direct masking than is touch, is thus further established. The experiments on the ***median*** and the ***ulnar*** nerves, wherein the thresholds were tested on the palm, point towards the same conclusion. But the experiments on the ***dorsal cutaneous nerve of the forearm*** yielded opposite results of comparable clarity. Furthermore, .the results obtained from the dorsal branch of the ulnar nerve, together with those from the medial and the lateral cutaneous nerves of the forearm, manifest heterogeneity meriting discussion.

The combined results from the four subjects for the *dorsal branch of the ulnar nerve* suggest greater elevation of the pressural threshold than of the tactual threshold, but the corresponding figures in columns 5 and 6 show discrepancy and lack of significance. Segregation of the results from one subject leaves those from the other three in excellent agreement and highly significant. Moreover, the corresponding figures in columns 5 and 6 show that the divergent results from the odd subject are in themselves inconsistent and insignificant. Hence we feel justified in ignoring them, and in accepting the conclusion so plainly indicated by the results from the other three subjects, that in this nerve the current tended to elevate the threshold for pressure higher than that for touch. The combined results from the three subjects for the *lateral cutaneous nerve of the forearm* suggest greater elevation of the tactual threshold than of the pressural threshold, but again columns 5 and 6 indicate inconsistency and doubtful significance. Segregation of the inconsistent and insignificant results from *the same* odd subject once more leaves the remainder reasonably consistent and highly significant, indicating that in this nerve the current tended to raise the threshold for touch more than that for pressure. In the experiments on the *medial cutaneous nerve of the forearm*, neither the combined nor the segregated results were satisfactorily consistent or clearly significant, though the combined data from two of the three subjects *suggest* a tendency towards higher elevation of the pressural threshold than of that for touch. For each nerve the subject whose results had to be segregated was the same, and in none of these instances were his results consistent or significant in themselves; moreover, the observations on this subject accorded with the rest in four of the seven nerves; and we all agree that accurate subjective standards of response are more difficult to establish in the forearm than in the hand. The discrepant observations do not diminish our confidence in the others; the following remarks consider only the latter -i.e., the significant results for each nerve.

These observations indicate that the alternating currents applied to the nerves investigated elevated the thresholds for pressure and for touch in the corresponding peripheral areas as follows: pressure higher than touch in the radial, the median, the ulnar (both on the palm and on the back of the hand), and (doubtfully) the medial cutaneous nerve of the forearm; touch higher than pressure in the lateral and the dorsal cutaneous nerves of the forearm. Certainly the general tendency is for pressure to be raised higher than touch in the hand, and touch higher than pressure in the forearm. In this connection it may be pointed out that although the hand is commonly thought of as a great tactual organ, much of what we loosely call touch is really pressure, as in clinical palpation. We acquire pressural information about external objects mostly through the hand, and sensory impressions from the hand contribute more than those from any other part of the body (except, of course, the special sense organs) to the mental cognition of external objects. The importance of sensory impressions from the hand in the upbuilding of the human mind has been emphasized by Wood Jones (1918, 1941), Wood Jones and Porteous (1928), and Elliot Smith (1931). Masking of common sensations being, as demonstrated experimentally by Thompson (1935b, 1943) a central phenomenon, and the feeling of pressure being an integrated mental concept, the outstanding susceptibility to masking manifested by pressure in the hand may well be associated with (*a*) the extensive central representation of the hand, demonstrated clinico-pathologically by Head (1918, 1920) and experimentally by Penfield and Boldrey (1937), and (*b*) the elaboration and refinement of the pressural component of manual stereognosis. Cognitively speaking, pressure is paramount in the hand, and the hand looms large in the brain.

SUPERFICIAL AND DEEP PRESSURE

Upon strongly stimulating a cutaneous nerve, we have always observed a rise in the peripheral pressural threshold, but rarely above 175 grams, using the technique described by Thompson *et al.* (1934). Similarly, we have observed that blocking a cutaneous nerve with procaine (for technical details see Thompson, 1943) seldom raised the pressural threshold over 175 grams. Evidently this represents approximately the limit of superficial pressure—i.e., pressure mediated through cutaneous nerves. This differentiation between superficial and deep pressure enabled us to study experimentally these two varieties of the pressural sense. Preliminary mention of these experiments has been made by Thompson (1935a): they are here reported and discussed in detail

1. Blocking the ulnar nerve with procaine and then strongly stimulating the radial nerve raised the pressural threshold in the "clear" radial area (as specified by Thompson, Luce, and Denny, 1948) so high that attempts to identify it were vitiated by slight movements of the hand caused by the heavy pressures applied; this result was obtained 16 times (two subjects). Thus blocking a deep

nerve and masking the sensation transmitted through the cutaneous nerve supplying the skin over a muscle supplied by the blocked deep nerve abolished all ordinary degrees of the pressure sensation in that part of the hand.

2. The converse experiment of blocking a cutaneous nerve and stimulating a deep nerve yielded a similar result, though the rise in threshold was not quite so uniformly excessive: the radial was blocked and the ulnar stimulated 40 times (four subjects), with significant masking of deep pressure 34 times (85.0 ± 5.6 per cent); the dorsal branch of the ulnar was blocked and the ulnar stimulated at the wrist 18 times (two subjects), with significant masking of deep pressure 17 times (94.4 ± 5.4 per cent).

3. As a control on the last type of experiment, the ulnar nerve was strongly stimulated at the wrist without any nerve being blocked, and pressural thresholds were taken in the radial and dorsal ulnar areas. This was done in the radial area 38 times (three subjects) without a single significant rise in threshold. In the dorsal ulnar area it was done 54 times (three subjects); 45 times there was no masking (83.3 ± 5.1 per cent); we feel justified by other experience in attributing the masking that occurred on the remaining nine occasions to accidental stimulation of the dorsal branch of the ulnar. We conclude that superficial pressure was not masked by stimulating the nerve supplying the underlying deep structures.

That the cutaneous tactual sense is not masked by stimulating any nerve other than that supplying the area tested is obvious; for if it were, the work of Thompson, Inman and Brownfield (1934) on tactual cutaneous nerve areas would have been impossible.

4. That superficial pressure similarly is not masked by stimulating any nerve other than that supplying the area tested is clearly shown by the results in Table III.

5. That superficial pressure is masked only by stimulating the nerve supplying the cutaneous area pressed upon was further tested by blocking the dorsal branch of the ulnar nerve and measuring the pressural threshold in the ulnar area of the palm with the following nerves under stimulation (three subjects in each instance): median, ten times; radial, medial cutaneous, and dorsal branch of ulnar (proximal to block), six times each. In no instance did a significant change in the pressural threshold occur in the palm. The point here is that the threshold for superficial pressure in the (clear) area of a nerve was not elevated by stimulating another nerve, even though a third nerve were blocked.

6. A final set of observations, to test this matter under yet other

experimental conditions, was obtained thus. The ulnar nerve being blocked at the wrist, the pressural threshold was measured in the (clear) radial area (*a*) with the median nerve under stimulation (16 times, two subjects), (*b*) with the medial cutaneous stimulated (four times, one subject), (*c*) with the *opposite* radial nerve stimulated (four times, one subject): on no occasion was the threshold significantly elevated. This indicates that abolition of deep pressure in subjacent structures by a procaine nerve block did not render the superficial pressural threshold liable to elevation by stimulation of any nerve other than that supplying the area pressed upon.

TABLE III

The figure after the stroke is the number of times the threshold for superficial pressure was measured; the figure before the stroke is the number of times it was elevated Each set of results is derived from experiments on three subjects

	Area in which Threshold was Measured			
Nerve stimulated	*Radial*	*Dorsal branch of ulnar*	*Ulnar in palm*	*Medial cutaneous*
Radial	39/39	0/22	0/19	—
Dorsal branch of ulnar	0/18	51/51	0/26	0/10
Ulnar at wrist	0/38	9/51	34/34	0/10
Ulnar at elbow	—		-	0/10
Median	0/20	1/30	0/36	—
Medial cutaneous	0/26	0/26	0/18	—

Thus it is clear that superficial tactual and pressural sensations can be masked only by stimulating the nerve supplying the area tested; whereas Thompson (1943) has shown that deep pressure in the hand can be masked by strongly stimulating *any* nerve in the forearm or hand. This was interestingly demonstrated in two of the incomplete nerve blocks mentioned but not described in that communication. In these unsuccessful attempts to block the ulnar nerve, its dorsal cutaneous branch was completely blocked, but its palmar portion, supplying deep structures, was only partially blocked, leaving some perception of deep pressure beneath the anaesthetized dorsal ulnar area; this residual deep pressure was masked by stimulating any nerve of the hand or forearm (the median, the radial, and the medial cutaneous nerve of the forearm were stimulated).

This striking difference in susceptibility to masking manifested by superficial and by deep pressure merits discussion in terms of the ideas (*a*) that masking is a central phenomenon and (*b*) that pressure is a mental concept.

It may reasonably be supposed that afferent fibres from superficial structures (skin and subcutaneous tissues) are connected with perceptive centres not completely identical with those receiving impulses from deep structures, such as muscles, ligaments, and periosteum; for brevity these may be referred to as "superficial" and "deep" centres respectively. Masking being a dulling of the perception of the sensations aroused by particular stimuli, resulting from the preoccupation or diversion of consciousness by the sensations aroused by other stimuli (Thompson, 1935b, 1943), it seems from the above experiments that the deep centres are very much more easily obfuscated than the superficial centres. For the perceptive faculty of the centre to which a cutaneous nerve, such as the radial, is connected can be dulled only by impulses ascending primarily to that centre via that nerve; Thompson, Luce, and Denny (1948) have pointed out that this bespeaks some measure of discreteness of the centres receiving impulses from the various peripheral nerve areas. Furthermore, Thompson, *et al.* (1931) have adduced evidence indicating that masking to any particular component of superficial sensation (e.g. pain) occurs only if the fibres mediating that modality are stimulated. Hence to dull perception of a particular sensation by a superficial centre it is necessary to stimulate not merely the nerve primarily connected to that centre, but the appropriate fibres within that nerve—probably fibres coming from specific end-organs. Thus the superficial centres seem to be highly organized in respect to the representation therein of the corporeal periphery, since in such experiments they can be dulled only by impulses ascending along groups of fibres specific not only in respect of the anatomical area whence they come, but even in point of their functional associations, and possibly of their end-organs.

In contrast, deep centres (at any rate those subserving pressure) may be dulled also by impulses ascending along fibres not primarily connected to those centres at all—impulses presumably only secondarily relayed (or "overflowing") to deep centres from superficial centres or from other deep centres. Now such deep centres receive impulses from muscles, ligaments, etc., impulses ordinarily initiated by proprioceptive stimuli, to which such centres may possibly be finely attuned and specifically responsive—we have not tested this. Even the pain of muscle-cramp might possibly be considered proprioceptive. We have studied the dulling of deep centres to almost the only exteroceptive stimulus that muscles normally experience, namely, deep pressure, and have found them strikingly susceptible.

All this conforms to the concept of the "body-image." Sensations are not merely felt, they are felt somewhere in the body. But the localization of sensations is a mental process, and they are localized not in terms of the actual body but in terms of the image of it that we have in mind. Vision being our dominant sense, the body-image is an image of the body *as we see it.* But practically the only part of our bodies that we see is the skin; the body-image is virtually an image of the contour of the skin, deeper structures therefore not appearing in the image. Superficial structures (skin, etc.) are connected with centres much more highly organized than those of deep structures (muscles, viscera, etc.). This is why sensations are so much more accurately localized in superficial than in deep parts of the body; why pain is referred from deep to superficial structures so much oftener than from superficial to deep; and why deep pressure is so much more susceptible to masking than is superficial pressure.

SUMMARY

1. In 20 experiments, involving three nerves and four human subjects, cutaneous tactual stimulation effected reversed masking to electrical stimulation of the nerve supplying the area touched; whereas in 18 experiments on the same nerves and subjects peripheral pressure failed to produce reversed masking.

2. These experiments are considered to support the idea of pressure as a mental concept developed from a spatial pattern of sensory impulses, rather than as a primary sensation. Other observations bearing on this are discussed; and this hypothesis is thought to fit a more extensive array of facts than the other. If this is correct, then the *specific* anatomical mechanism responsible for the feeling of pressure lies not at the periphery but in the brain.

3. Analysis of 146 observations, involving seven nerves and seven subjects, indicates that (despite some inconsistencies, considered negligible) in the hand pressure is more susceptible to direct masking by electrical stimulation of a nerve than is touch, whereas in the forearm pressure is the less susceptible.

4. This is attributed to the specialization and cognitive importance of the pressural component of manual stereognosis.

5. A considerable number and variety of experiments reported herein indicate that deep pressure is much more susceptible to direct masking than is superficial pressure.

6. This differential susceptibility of superficial and deep pressure is discussed in relationship to the nature of the body-image.

REFERENCES

ADRIAN, E. D. (1928). The basis of sensation (London, Christophers).

BAZETT, H. C. (1935). Methods of investigation of sensation in man and the theoretical value of the results obtained; in Sensation: its mechanisms and disturbances, edited by C. A. Patten, A. M. Frantz and C. C. Hare, Research Publications of the Association for Research in Nervous and Mental Disease, vol. XV, chap. IV, pp. 83-97. Baltimore, The Williams and Wilkins Co)

HEAD, H. (1918). Sensation and the cerebral cortex. Brain, **41**: 57-253.

——— (1920). Studies in neurology. Oxford Medical Publications.

HEINBECKER, P., BISHOP, G. H. and O'LEARY, J. (1934). Analysis of sensation in terms of the nerve impulse. Arch. Neurol. & Psych., **31**. 34-53

JONES, F. W. (1918). Arboreal man. E. Arnold.

— — (1941). The principles of anatomy as seen in the hand Baillière, Tindall, and Cox.

——— and PORTEOUS, S. D. (1928). The matrix of the mind. University of Hawaii Press Association.

PENFIELD, W. and BOLDREY, E. (1937). Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain, **60**: 389-443.

SHEEHAN, D. (1933) The clinical significance of the nerve-endings in the mesentery. Lancet, **1**: 409-13.

SMITH, G. E. (1934). Evolution of the mind. Nature, **133**: 245-52 (supplement, Feb. 17).

THOMPSON, I. M. (1933). The effects of alternating currents upon cutaneous sensory thresholds. Science, **78**: 268-9.

— — (1935a). An outline of the application of certain technical advances to some problems of cutaneous innervation and sensation, in Sensation, its mechanisms and disturbances, edited by C. A. Patten, A M. Frantz and C. C. Hare, Research Publications of the Association for Research in Nervous and Mental Disease, vol. XV, chap V, pp. 98-102. Balitmore, The Williams and Wilkins Co.

——— (1935b). Sensory masking—a peripheral or a central phenomenon? Science, **82**: 221.

——— (1943). An experimental anatomical study of sensory masking; in Essays in biology in honor of Herbert M. Evans, pp. 555-9. University of California Press.

——— BANKS, G. F., BARRON, A., FRATIS, A. M. JR., and MATTISON, B. F. (1934). Differential elevation of cutaneous sensory thresholds by alternating currents applied to a nerve. Univ. Calif. Publ. in Anat., **1**: 167-94.

——— INMAN, V. T. and BROWNFIELD, B. (1934). On the cutaneous nerve areas of the forearm and hand. Univ. Calif. Publ. in Anat., **1**: 195-236.

——— and KIMBALL, H. S. (1938). Has the sensation of pressure its own peripheral anatomical mechanism? Anat. Rec., **70**: 78 (suppl. 3).

——— LUCE, J. C., and DENNY, L. W. (1948). Experiments on human cutaneous sensory thresholds. Trans. Roy. Soc. Can., series III, sec. V, **42**: 165-174.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIII : SERIES III : JUNE, 1949
SECTION FIVE

FLAVELLE LECTURE

Some Problems in the Cytogenetics of Cereals: A Historical Review

By W. P. THOMPSON, F.R.S.C.

I. The Solution of the Rust Problem

THE first major research project which I undertook after I had to stand on my own feet, was a systematic investigation of the genetics of wheat, an obvious project for a geneticist in Saskatchewan. It soon came to include the genetics of rust-resistance and thereby became involved in the whole problem of wheat rust. The ultimate solution of that problem through the contributions of many workers not only conferred immense economic benefits on the people of Canada but also led to several fundamental scientific advances. The whole enterprise was a model of scientific co-operation and of intelligent leadership and supervision. It is therefore important from the historical standpoint that the story be told with scientific accuracy.

Nearly all those who were prominent in the first phase of the work and who could therefore tell the whole story are now dead: Buller, Fraser, Saunders, Murray, Tory, MacLean, Grisdale—four of them Fellows of this Society, two Presidents, and two medallists. Almost the only survivors from the first group are John Bracken and the writer, and Mr. Bracken long ago developed other and more absorbing interests. Since I am also about to assume other duties I shall devote a portion of this scientific swan-song to discharging the duty of historian.

In 1916 the first large-scale epidemic of stem rust of wheat in western Canada destroyed many millions of bushels of wheat which were badly needed in a war-torn world. If rust had not been present the crop would have been enormous, and those western portions of the prairie to which the rust did not spread enjoyed very high yields. Similar experiences were repeated in many subsequent years.

Following that first epidemic Doctor Walter Murray, then President of the University of Saskatchewan and member of the recently founded National Research Council, on the advice of his professors of field husbandry and biology, took the initiative in urging a co-operative attack on the problem. After much indifference and inertia had been overcome, a conference was convened in Winnipeg in August 1917. It included the presidents and professors of botany and field husbandry of the prairie universities, the Director of the Dominion Experimental Farms and certain of his superintendents, the Dominion Cerealist and his assistant, and a Dominion Plant Pathologist. Almost the whole time of the conference was devoted to such proposed measures as tillage practices, the eradication of the barberry which is the second host of the rust organism, the development of early varieties which might be expected to ripen before the rust attack became too severe, and studies on the life history of the rust in the hope of discovering some vulnerable point.

Scarcely any attention was devoted to the possibility of producing rust-resistant varieties by genetical methods. The proposal that attempts should be made to produce such varieties was made to the conference by the writer and was based on the fact that during the great epidemic of 1916 two agronomically useless varieties which I had been growing were almost free of rust although completely surrounded by varieties which were severely attacked. Both these varieties belonged to species which are quite different from that to which bread wheat belongs; one was *Triticum durum* and the other *T. dicoccum*.

The proposal aroused very little interest. In fact only two brief comments were made. One member of the conference expressed the opinion that it was useless to expect anything worthwhile from a cross between a bread wheat and a *durum* or *dicoccum*. He had made such crosses for another purpose and the hybrids were extremely unpromising for several reasons. The second comment was support for the proposal from the late Professor W. P. Fraser, then an officer of the Dominion Department of Agriculture, who thought that the attempt should be made on the principle that every possibility should be explored. But so little was thought of the proposal that it is not even mentioned in the official minutes of the conference.

In view of the subsequent success of the breeding procedure, it is difficult nowadays to realize that the production of rust-resistant varieties by genetical methods was a new idea at that time, and that to many it seemed a very unpromising idea. But it must be remembered that there were then not more than two or three trained geneticists in the whole of Canada. And very few technical agriculturists, even those who would be called plant breeders, understood the elementary principles of Mendelism. Therefore the conference concentrated on the other measures which were mentioned above. They said in effect "You can go ahead if you like but it looks to us like a waste of time and we will depend on other methods."

The attitude and hopes of the conference can be judged from the two main resolutions which it passed. One of these dealt with the purely pathological phases and the other with all the remaining phases of the work planned. The latter reads as follows:

> Resolved that we, the delegates to this conference assembled, in view of the very serious damage caused by rust in our otherwise most favorable seasons, recommend that investigation work for the determining of methods of crop and soil management best suited to lessen the damage from this fungus be instituted by the Dominion Department of Agriculture on each of its branch farms and stations in the Prairie Provinces and by the Universities, Agricultural Colleges and Schools in each of the Prairie Provinces.
>
> We further recommend that this work include the following lines of investigation:
>
> 1. Influence of different dates of seeding.
> 2. Influence of different rates of seeding.
> 3. Influence of different times of cutting.
> 4. Influence of different methods of tillage.
> 5. Influence of different methods of fertilizing.
> 6. Influence of different preceding crops.
> 7. Observations on the rust resistance of different varieties.

Two committees were set up to supervise the work which was planned under these two resolutions. It was agreed that all institutions represented would co-operate in these undertakings and that the Dominion Department of Agriculture would appoint staff to work in laboratories and greenhouses provided by the provincial institutions. In this way the Dominion Laboratories of Plant Pathology were established at the Universities of Saskatchewan and Alberta and have continued to the present.

During the next few years much information was collected and two outstanding discoveries affecting the breeding of resistant varieties were made. One was the existence of numerous strains of the rust organism which differ only in their capacity to attack different varieties of wheat, so that a variety which is resistant to the rust strains present in one year or place might not be resistant to those of another year or place. These rust strains are now numbered in the hundreds.

The second outstanding discovery was that the different species of wheat have different chromosome numbers. Thus bread wheat (*Triticum vulgare*) which at that time included no resistant varieties, has 42 chromosomes (diploid) and those species from which it was hoped to derive resistance (*T. durum* and *T. dicoccum*) have 28. It was just beginning to be realized that the determiners of hereditary characters such as rust-resistance are located in the chromosomes. (The first text-book which accepted this theory was published in 1919.) It was therefore suspected that the difference in chromosome numbers between the species which had to be crossed was responsible not only for the partial sterility of the hybrids but also for the profound disturbance or complete breakdown of the Mendelian principles on which the breeding procedure was based.

These two discoveries revealed the unsuspected difficulties and complications of the problem, and showed that a great deal of fundamental work on rust strains and chromosome behaviour would have to be done before a practical solution could be expected.

Nevertheless by the time a second conference was called in 1924, following another disastrous epidemic in 1923, sufficient progress had been made both in Canada and in the United States to convince everyone that the best method of reaching a practical solution of the problem was the production, through cross-breeding procedure, of varieties which would be rust-resistant and at the same time would possess the other necessary agronomic qualities. All hopes became based on the development of such varieties. Important as all the other work might be from the scientific standpoint, it would from the practical standpoint only clear the way for and assist the breeders.

This second conference was called by Dr. H. M. Tory as President of the National Research Council and Dr. J. H. Grisdale as Deputy Minister of Agriculture and was held in Winnipeg in September, 1924. All scientists from the prairie provinces, the National Research Council and the Federal Department of Agriculture who might be expected to contribute were invited. By this time there had been a great increase in the number of trained research men in these services and in the available and prospective funds. The conference was therefore able to make comprehensive plans to attack all phases of the problem and to assign each phase to one or more workers who were specially qualified to carry it on.

The Dominion Department of Agriculture undertook to establish a Rust Research Laboratory at Winnipeg. This was soon built and well staffed with experts in plant pathology and a group of able plant breeders. To it was assigned much of the pathological work and all the work on the recently discovered rust strains. Professor Buller of the University of Manitoba and Professor Fraser of the University of Saskatchewan undertook particular phases of the pathological and mycological work. Trained geneticists were now available for the breeding work not only at the Rust Laboratory (Goulden, Neatby) but also at the prairie universities (Roberts, Wiener, Elder, Harrington, and Aamodt). Each of them was assigned a group of the most promising crosses, so that an immense amount of breeding work could be done without duplication. The chromosome situation and behaviour and their relation to breeding results arising from the differences in chromosome numbers in different species of wheat, were assigned to the writer and his associates who had already made some progress in this area. Biochemical studies on the nature of resistance were obviously needed and were undertaken by Dr. (now President) Robert Newton of Alberta. Full provision was made for the agronomic study and assessment of prospective varieties at the prairie experimental stations and at Ottawa. It was realized also that careful studies of the milling and baking qualities of the new varieties would have to be carried on and arrangements were made for the co-operation of a series of laboratories including those of the Dominion Cerealist, the Grain Board, and the Chemistry Departments of the prairie universities.

Thus through the far-sighted leadership of Dr. Tory, Dr. Newton as Chief of the Division of Applied Biology of the National Research Council, and Dr. Grisdale, Deputy Minister of Agriculture, and with the enthusiastic co-operation of many kinds of scientists in provincial and federal institutions, a comprehensive attack on all phases of the problem could be undertaken. The Associate Committee on Cereal Grain Rust of the National Research Council was set up under the joint chairmanship of the President of the Council and the Deputy Minister of Agriculture, to supervise the work without restricting the initiative of the individual workers. Annually thereafter all workers met in conference to report their progress, to see how each man's results affected the work of others, and to modify plans in the light of that progress. The provincial workers were generously supported by the Research Council and the Dominion workers by the Department of Agriculture. Eighteen different persons received grants—most of them several grants—from the Research Council through this committee.

As a direct outcome of this work satisfactory rust-resistant varieties began to take their place on the farms during the nineteen thirties, in friendly competition with a similar variety produced by similar methods at Minnesota. At present approximately 90 per cent of the hundreds of millions of bushels grown annually in western Canada belongs to these varieties, the rest being grown in western Alberta where rust was never a serious menace. Improvements in the original resistant varieties with respect to other qualities are constantly being made by genetical methods.

The rust problem is now solved. It may not stay solved because strains of rust which can attack the present resistant varieties are known and may spread. But we may rely on those who defeated the enemy before to defeat it again. As a matter of insurance they are already taking measures to produce varieties resistant to the newer strains of rust. The struggle may have to be carried on for a long time.

The enormous economic benefits flowing from this work should be emphasized. The average annual production of wheat on the prairies from 1940 to 1944 was 103 million bushels with an annual value of 334 million dollars. In the single year 1946 the value of the wheat crop was 460 million dollars. Even if the greatest possible allowance is made for the yield which might have been expected if only the older, non-resistant varieties had been available, it is easy to see that the gain is tremendous. No doubt in many single years the gain would more than pay the total expenditure of the Research Council since its establishment. Nor may we forget the individual farmer who had to look on helplessly while a promising crop on which he depended was destroyed before his eyes.

But the benefits were not confined to the defeat of stem rust of wheat. The organization set up to fight that disease has dealt with and is now dealing with other diseases of wheat and other crop plants—smuts, foot rots, root rots, take-all, etc. The organization, the method of attack, the co-operative spirit, the personal associations, are proving as valuable for these newer projects as for the original problem.

Nor were the advances confined to the practical economic field. Several members of the group made fundamental scientific discoveries of note in the course of the co-operative enterprise. Dr. Craigie's discovery of sex in rust and the details of its operation earned him a world-wide reputation and many marks of recognition, including the Flavelle Medal of this Society. Several of the late Professor Buller's fundamental studies grew out of the work of the group and this Society now has in the press the last volume of his researches in this field. The discoveries of Dr. Margaret Newton and Dr. T. Johnson in relation to the rust strains, their number, origin, relationships, differences, and inheritance, are well known and were recognized last year by this Society through the award of a Flavelle medal to Dr. Newton. The work of the breeders, notably Goulden and Neatby, in analyzing the genetics of rust resistance as well as in producing satisfactory varieties, was a fine achievement. The writer and his students were able to throw some light on the behaviour of the chromosomes in interspecific crosses and its relation to sterility and genetic behaviour.

The whole enterprise is an excellent example of co-operation in research. It involved the combined efforts of several plant pathologists each working on his own phase of the problem, a group of breeders amongst whom were divided the most promising crosses, agronomists, cytogeneticists, biochemists working on the nature of resistance and cereal chemists working on milling and baking qualities. These investigators were located in several different institutions from Edmonton to Ottawa and in different departments of individual institutions. It is known of course who carried out the particular crosses which produced the varieties now used but the happy outcome is the result of the combined efforts of the whole group and all deserve a share of the credit. Of particular importance was the very high order of leadership and supervision which encouraged the enthusiasm of all concerned and tactfully directed it into productive channels without destroying initiative.

II. OTHER PROBLEMS

The work on the genetics of rust-resistance soon involved the problem of hybrid sterility and its genetic effects, because it was necessary to cross two distinct species and the hybrids produced were partially sterile. My associates and I were able to identify the immediate causes of the partial sterility and to assess the precise effect of each. On the male side the following immediate causes were involved: (1) complete abortion of some pollen grains, (2) retardation of development of others, (3) failure to germinate, (4) inability to function though capable of germinating. On the female side there was almost no pre-fertilization breakdown, nearly all embryo-sacs proving capable of fertilization. By far the most important cause of sterility was the abortion of the endosperm which in turn caused the death of many hybrid embryos.

Behind these immediate causes of sterility and responsible for them is the basic chromosome situation involving numbers, behaviour, and incompatibility. Bread wheat has three sets of seven chromosomes (haploid) and the emmer species two sets of seven. The behaviour of the chromosomes and its effects are complicated in detail but clear in general outline. It was possible for us to show that the sterility is due basically to the inviability of many chromosome combinations and that this is governed by rules which were formulated. For example the farther the chromosomes of the endosperm depart from the complete absence or complete triploidy of the distinctive *vulgare* set of 7, the more severe are the disturbances leading to abortion.

In this connection a result of general interest and importance is that when two species which differ in chromosome number are crossed, the cross is usually more successful (often much more) if the species with the larger number is used as the female parent than if it is used as the male. This was also elucidated on the ground of chromosome behaviour. It proved to be of wide application in the plant kingdom.

The chromosome situation and behaviour which causes the sterility was shown to be chiefly responsible also for the very distorted genetics and great difficulties in a breeding programme which involves interspecific crosses. The essential feature is that Mendelian recombination of the parental characters is largely interfered with, so that most segregates exhibit the whole group of characters of one or the other species. The absent (and often desired) combinations of characters are missing because the combinations of chromosomes necessary to produce them are inviable.

From the original crosses involving fairly closely related species and only partial sterility, the work was extended to involve more distantly related species of wheat and then to crosses between wheat and rye, *Aegilops*, and *Agropyron*, and between rye and barley. These constitute an extraordinary series of intergeneric crosses. In general, fertilization can be brought about with no great difficulty in these very wide crosses, and if breakdown in the reproductive processes occurs, it is almost always due primarily to abortion of the endosperm. This may cause the death of the embryo and the failure of the cross.

In those cases in which the hybrid embryo is able to develop to maturity, the study of the chromosomes at the meiotic division of the hybrid yields much valuable information not only about the mechanics of chromosome behaviour but also about chromosome homologies and evolutionary relationships and processes in this large group of species. In order to understand these results it must be kept in mind that all the species of all these genera have their chromosome numbers in multiples of seven. Thus two species of *Triticum* have only one haploid set of seven, eight species have two sets of seven, and four species have three sets. There is a similar situation in the other genera, the number of sets in *Aegilops* varying in different species from one to three, and in *Agropyron* from one to five, while *Secale* has only one set. Studies on the mating of the chromosomes in various hybrids enables us to draw such conclusions as the following: (1) The single set in 7-chromosome wheats is also present in those species of wheat which have two sets. (2) The two sets of the latter are also present in those which have three sets. (3) One wheat set is present in certain species of *Aegilops*. (4) At least one wheat set is found in *Agropyron*. The different sets in various species of all these genera have been designated by symbols and their homologies determined. The results have thrown much light on the evolutionary relationships within the whole group and on the role of hybridization and chromosome doubling in evolution.

From studies of this kind many of the characters whose primary genes are located in particular sets of chromosomes have been determined. But the localization of the genes has been carried much farther than that. Different segregates from crosses between species which differ by one set, lack different members of that set. A study of such segregates may reveal the particular chromosome in which certain genes are located. In addition Sears has utilized the occurrence of monosomics and their derived nullosomics to identify many of the individual chromosomes of common wheat which carry particular genes.

From results of the type described it was concluded that the 21-chromosome bread wheats had originated by a natural cross between a 14-chromosome (emmer) species of wheat and a species of the genus *Aegilops*. The reasons for this conclusion are: (1) that the characteristics which distinguish bread wheats from emmer wheats are also found in certain species of *Aegilops* and (2) those species of *Aegilops* have a set of seven chromosomes which appear to be homologous with a set in bread wheat. This would mean that two of the sets of seven chromosomes in *T. vulgare* have come from another species of wheat and the third set from *Aegilops*.

In order to test this hypothesis and at the same time to produce common wheat artificially, we crossed *Triticum turgidum* and *Aegilops speltoides*. The former has 14 haploid chromosomes and the latter 7 (28 and 14 respectively in somatic or diploid cells). The hybrid therefore had 14 + 7, and was completely sterile because of very irregular chromosome behaviour at meiosis. We then succeeded in doubling the chromosomes in a sterile hybrid by treatment with colchicine. Through this doubling each chromosome acquired an exactly similar mate and the meiotic divisions therefore proceeded normally. Fertility was immediately restored. The resulting plants of course had twice 14 + 7 or 42 chromosomes in their somatic cells. This is the number in bread wheat. They also had many of the distinguishing characters of bread wheat and proved to be fertile in crosses with natural bread wheat. It may therefore be concluded that an old and well-known and very important species has been artificially synthesized from its putative ancestors. A considerable number of new species within this group have since been artificially synthesized by Zhebrak, Sears, and others.

TRANSACTIONS OF
THE ROYAL SOCIETY
OF CANADA

SECTION V
BIOLOGICAL SCIENCES

THIRD SERIES—VOLUME XLIV—SECTION V

JUNE, 1950

OTTAWA
THE ROYAL SOCIETY OF CANADA
1950

TABLE OF CONTENTS

* * *

Parasitology and Evolution. Presidential Address by T. W. M. Cameron, F.R.S.C. 1

A Tetraploid Form of Annual Rape Induced by Colchicine. By J. M. Armstrong 21

A Study of the Mechanism of Reversal of Spinal and Bulbar Reflexes. By R. A. Hughes, G. W. Stavraky, and R. D. Teasdall 39

Relation entre le diagramme de solidification et la toxicité du système Diméthylamino-phényldiméthylpyrazolone–Bromodiéthylacétylurée. Par Jules Labarre, M.S.R.C., et Louis Trochu. 61

A Consideration of Muskrat Populations and Population Trends in Manitoba. By J. A. McLeod 69

Specific Action of Chloromycetin in the Case of Pasteurella Felis Infection in Man. By J. Édouard Morin, F.R.S.C. . . . 81

Variations in the Silk-Spinning and Locomotor Activities of Larvae of the Spruce Budworm, Choristoneura fumiferana (*Clem.*), *at Different Rates of Evaporation.* By W. G. Wellington . 89

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIV : SERIES III : JUNE, 1950
SECTION FIVE

PRESIDENTIAL ADDRESS

Parasitology and Evolution[1]

By T. W. M. CAMERON, F.R.S.C.

PARASITISM is an ancient phenomenon, one which goes back well into geological time. But parasites leave no skeletons and attempts to trace their antiquity must rest partly on their modern anatomy and partly on the palaeontology of their hosts. This essay is an attempt to show that internal parasitism is and probably always has been part of the general pattern of life. It is not so much concerned with conclusions as it is with future potentialities; it tries to point out a pathway rather than to delimit a road, to erect signposts rather than milestones.

There is no really satisfactory objective definition of a parasite and in almost every case altogether too much weight is given to actual or potential pathogenicity. Attempts have been made on numerous occasions to classify dependent organisms as consors, symbionts, commensals, parasites, and other similar groups, but while these divisions are defensible on theoretical grounds, they too often break down in practice. Accordingly, for the purpose of this discussion a parasite is regarded as an organism which has adapted itself to live in a particular kind of environment— another organism—and is in consequence dependent on that organism for some essential substance; this is often an enzyme system or part of one and it has to be provided by the host.

Sometimes the host may draw some advantage from the association; at other times—even with the same parasite—the reverse may be true. The parasite lives in a living environment and its pathogenicity is merely the alterations in that environment caused by its presence. A virulent parasite is one which causes serious disease, that is, one which causes considerable alterations in the environment either because the environment is more easily altered than usual, its powers of regeneration are reduced, or some essential factors are absent; or because the activity of the parasite is greater than usual, its development is more favoured, or its metabolic products are more toxic; or because of any combination of these and similar factors.

[1] Contribution from the Institute of Parasitology, Macdonald College, McGill University, with financial assistance from the National Research Council of Canada.

None of these factors is important from the present point of view as it is obvious that a virulent parasite will tend to eradicate its host and consequently itself. Successful and, therefore, long-standing parasites tend towards avirulence, natural selection eliminating the virulent forms. The important facts are that the host supplies certain substances required for the parasite to live and that these substances can be supplied without destroying the host.

Now, the general effect of a static environment is to cause natural selection to check evolution rather than to accelerate it, as the variations which tend to depart most from the mean are rejected. The long-term environment of the parasite, however, is often not fixed but is subject to change as the host itself evolves. Accordingly, the physiological demands of the parasite must remain more plastic than the environment or the parasite will die and the species disappear. There is a limit to the plasticity of any organism, however, and many well-established parasites have, in the course of evolution, become extremely host specific. Some are able to live only in a single species of host, others in closely related forms; in a few cases this host specificity is almost absent. In general terms, host specificity often is a function of evolutionary age and specialization, and the more divergent a parasite is from its main phylogenetic stem, the more it tends to be host specific. Jean Baer has consequently recognized two types of host specificity:

(1) *Phylogenetic*, such as is observed in the taenoid cestodes and in many nematodes, in which only "natural" hosts can be infected. This type of specificity is very old and it is probable that the hosts already harboured worms at the time of the great evolutionary surge which descended on the terrestrial vertebrates at the end of the Mesozoic.

(2) *Ethnologic*, such as is observed in digenetic trematodes, in which the infection depends mainly on the nature of the food. It is probably a relatively recent adaptation at an epoch after the great groups of vertebrates were established.

Host specificity is a physiological attribute but in our present state of knowledge we have no ready method of recognizing its presence. Our best information comes from morphology and morphological differences. It is probably true that, in general, physiological differences precede morphological ones and need not necessarily be accompanied by any change in anatomy. It is also probably true that certain morphological changes in parasites have no connection with physiology and occur quite by accident; unnecessary features may or may not disappear if they do not affect the parasite's well-being. However, at present, morphology is our only source of information.

It is equally true, that while there are many more parasites than hosts in the world, only a very few have been studied and there are many gaps in our knowledge. Even with our present admittedly defective information, it is possible to draw certain generalizations from a taxonomic study of these parasites.

As host specificity probably depends on the suitability of the biochemical environment and as similar environments are most likely to occur in phylogenetically related hosts, their parasites are most likely to have related metabolic requirements. The more specialized both in habit and habitat the parasite, the more probable this will be, and it is sometimes possible to reconstruct from this kind of information the evolutionary background of the parasite itself. Related hosts tend to have related parasites. However, this generalization must be interpreted with care, because an examination of the parasite fauna of living vertebrates shows that it is also dependent on the past and present environment and habits of the host —factors which are also poorly understood.

All parasites must sooner or later, at some stage or other, leave the host to be transferred to a new host. The period outside the body may be short or long; it may require residence in an intermediate vector which may in due course inject the parasite into the host or be eaten by it. If no vector is involved, the parasite may re-enter the host as a faecal contamination of food or actively penetrate its skin. In many cases it must re-enter the wrong host and sometimes it may take up a semi-permanent residence in the wrong host. In fact, the accidental transfer of a parasite from one gregarious species to another probably represents the standard evolutionary story of most strictly parasitic animals. The more closely related the hosts, the more probable will be the chances of a suitable mutant being produced.

No parasite is capable of completing its full cycle outside of the host and to compensate for the difficulties so involved, all parasites have developed a high fecundity and either produce innumerable progeny within the host or have a special multiplication cycle outside of the host. The total parasite population must remain more or less stationary under normal circumstances; otherwise the parasites would die out because of the adverse effect of overcrowding in the host or because too few young are produced for successful re-entry. Accordingly, the chances of re-entering are inversely proportional to the number of offspring produced. This is true only under normal conditions, however, and a change of these conditions may completely alter the balance—as happened with the development of agriculture in the case of man and his domestic animals. A change in climate or in feeding habits of the host will also alter the parasite picture, and for this reason, even closely related hosts may have different parasite fauna.

A different set of difficulties arises from the difficulties in determining a species or a genus—difficulties which are inherent in taxonomy but are enormously multiplied in parasitic animals, which have frequently dispensed with many external characters. Even although one accepts the dictum that a species is a series of individuals more closely resembling each other than the individuals of any other species, in the last analysis, the concept of a species depends on a worker's own judgment. Individuals are functioning, reproducing organisms and dead specimens only represent such individuals. As has been pointed out, the species represents the result of an individual's effort to extend itself and, as we actually know it, is only the concept of a taxonomist developed on the basis of his own data. A genus also is but one man's concept of a group of species which, to him at least, appear to be more closely related to each other than to the species of any other genus.

This concept varies enormously, and often capriciously, with different taxonomists and very often a genus—as well as higher groups—is determined by the presence or absence of a single arbitrarily selected character. To add to these difficulties is the fact that many descriptions are inaccurate or insufficient—a trouble which is by no means confined to early writers. Often this is traceable to a lack of interest in parasites other than those causing disease in man or his economic animals. The amount of invaluable material which has been thoughtlessly discarded by zoologists is incalculable.

However, a great amount of information is already available and an examination of the parasites of Baer's phylogenetic host-specific groups—the sporozoa, the cestoda, and certain groups of nematodes—points to certain generalizations of considerable importance.

PARASITE PROBLEMS

The sporozoa. Among the sporozoa, the genus with the most interesting host distribution is *Plasmodium*, three species of which occur in man and cause the various malarias. These are of two types—quartan and tertian, which relapse and are caused by parasites with more or less circular gametocytes, and subtertian, which does not relapse and the parasites possess crescentic gametocytes.

Disregarding obvious erratics, *Plasmodium*-like parasites are confined to primates, bats, and insectivores, to birds, and to reptiles.

In addition to the three common species in man, *Plasmodium* occurs in the great apes, Old World monkeys, and New World monkeys. In the apes, the parasites are very close to, if not identical with those in human beings. At least four species occur in Old World monkeys and these are more distant from the human forms in morphology although one, in Malayan monkeys, is not unlike *Plasmodium vivax*. There is more uncertainty about the species in New World monkeys and although only one is recognized, it may represent more than one; it shows some resemblance to *P. malariae* of man. So far as is known, the primate species are transmitted by anopheline mosquitoes.

Species of *Plasmodium* are also found in bats, although they have not been thoroughly studied in these animals. Nor have they been completely studied in insectivores.

In birds, *Plasmodium* occurs in over two hundred species— mostly in passerine birds —and includes about five species of the *vivax-malariae* type and ten of the *falciparum* type. So far as is known, the vectors are culicine mosquitoes.

At least a dozen species of *Plasmodium* occur in reptiles and they exhibit a striking parallelism with the forms in birds; they have been reported mostly from lizards and snakes. One group produces elongated gametocytes, the other, round or oval forms; in the latter group one species shows only gametocytes in the peripheral blood, while of the remainder some produce few, others many merozoites. In all the reptilian species, however, the gametocytes tend to be more variable in shape than in the avian-mammalian forms and in some species they are intermediate in form between "elongated" and "round." They occur both in Africa and South and Central America.

The available evidence accordingly suggests that *Plasmodium* was originally a reptilian parasite, passing into birds and mammals when they left the reptilian stem but in the mammals being restricted to the evolutionary branch of the insectivores, the primates, and bats, with a few erratics in other groups.

The human malarial parasites have a straight family tree going back to the reptiles but their more recent distribution presents some difficult problems. While it is easy to follow the geographical spread of *P. malariae* and *P. vivax* (both of which can live in temperate climates), it is still difficult to trace the history of *P. falciparum*, which cannot persist outside of the tropics and sub-tropics. It has been known in the warmer parts of the Old World since the time of written history. If *P. falciparum* were present in South America on Columbus's arrival, as is usually believed, it is difficult to account for its presence there except by parallel evolution from cebid monkeys. It could not have withstood the rigours of a long slow passage down North America through Siberia from tropical Asia. Its short life span would probably militate against even a voyage from one tropical Pacific island to another and finally to the mainland. The different strains of *P. falciparum* may prove to be different evolutionary lines from pre-falciparum forms in early cebid and early Old World monkeys and apes which were following out the lines so clearly stamped on reptilian and avian forms. Of course, *falciparum* malaria may not have been present in South American men until it was imported from Africa in post-Columbian times, but even then, much the same difficulty in accounting for its presence still exists.

Nematodes. While certain groups of parasitic nematodes can be recognized as containing forms so closely related as to suggest a common origin, it is often difficult to trace any close relationship with other groups and at present it is impossible to formulate a true system of classification. Even our best attempts are based frankly on arbitrarily selected characters and are a basis for future discussion and rearrangement only when more forms are known, existing forms more accurately described, and more extensive studies made on life cycles and biology. However, there is little doubt that the parasitic forms are polyphyletic in origin. Only a very few of these can be discussed here.

Probably the Oxyurids or "pinworms" represent the least unaltered morphologically of the parasitic nematodes, although in their life cycles and host specificity, they are among the most specialized. Undoubtedly they are very old parasites which are probably still inhabiting their original habitat, the large bowel, and feeding on its contents; for this purpose they have retained the typical "double-bulb" type of oesophagus characteristic of free-living feeders on bulky material. This consists of a club-shaped anterior portion with the usual triangular lumen, a narrow neck, and a posterior spherical portion with radial fibres and a central double valvular apparatus which greatly increases its sucking capacity. Such an oesophagus is an ideal sucking mechanism for species living in the lumen of the large intestine and no special modification is required. However, the life cycle of these forms became very intimate. An egg, partly embryonated when passed in the faeces, quickly becomes infective and is ingested by the host; it need only be outside of the host for at most a few hours. Such a parasite tends, in consequence, to be confined to closely related individuals and consequently to evolve with them. An examination of the described forms (if we exclude such aberrant forms as *Oxyuris equi* of the horse)[2] shows that most occur in insects and reptiles with a fairly large group in rabbits and rodents, and one genus each in primates, in marsupials, and in "flying lemurs."

---

[2] There seems no explanation for the genus *Oxyuris* in this series. It is far from typical of the family of which it is the type in both anatomy and development. Its fourth-stage larva is a blood-sucking form and its resemblances to the other oxyurids may well be due to convergence rather than to parallel evolution. No other oxyurids are known from either the carnivores or the ungulates.

The genus in primates is *Enterobius*, species of which genus occur in man, the great apes, the Old World monkeys, the New World monkeys, and the lemurs. These species tend to occur in genera of hosts or even, as in South American monkeys, in groups of related genera..Moreover, the extent of the morphological differences between the species of pinworms varies directly with the differences between hosts. The evidence strongly supports the thesis that *Enterobius* was originally a proto-primate parasite which has evolved along with its host but at a somewhat slower rate.

A few pinworms have been described from Australian marsupials and although they are imperfectly known they resemble those seen in rabbits. However, in South American marsupials, there occurs a very specialized genus, *Cruzia*, which, although still obviously an oxyurid, is quite unlike the other species in mammals. The oxyurid of the flying lemurs of Asia is also a very specialized form.

Resembling the oxyurids proper is a family of viviparous species – the Atractidae—members of which occur in fish, reptiles, and various mammals including horses, hippopotami, elephants, and rhinoceroses. All are small forms, sometimes with a very ornate mouth, and the female has a single ovary (two in the case of *Probstmayria* of the horse). They are parasites of the intestine probably always the large intestine and may be able to complete their entire life cycle therein. It is highly probable that many more members of this group await description and they may well be an independent group which has passed from a free-living existence directly to life in the lumen of the large bowel of cold-blooded vertebrates and from them into the rather specialized orders of ungulates in which they occur.

Closely related oxyurids occur in the rectum of various insects. These are more specialized than the species in vertebrates and possess characters which suggest that they have developed a more elaborate type of life history. However, as little experimental work has been carried out with them, they cannot be considered here.

An entirely different series of nematodes is found in the whipworm group, its most primitive genus being *Capillaria*, many closely related species of which occur in a wide variety of mammals, birds, reptiles, and amphibia. It is one of the commonest nematode parasites. Like other members of the group, it has a very small, degenerated, club-shaped oesophagus and a well-developed series of glands surrounding the tube joining this oseophagus to the intestine. Most species are inhabitants of the small intestine but some live elsewhere in the digestive tract as well as in the urinary bladder, liver, and lungs. The female has a single ovarian tubule and produces eggs with characteristic polar caps. The typical life cycle involves a lengthy development period within the egg outside of the body and a return to the host still within the egg.

From this primitive stem have evolved some very specialized forms. *Trichinella spiralis* is really a viviparous *Capillaria* living in the small intestine. Its larvae encyst in the flesh of the original host and are carried to the next host only when it eats the original one. Consequently, it is a parasite of carnivores and this development cycle has tended to confine its natural distribution to holarctic boreal regions where carnivores habitually eat carnivores. However, it has no natural specificity for mammals and will live in practically any to which it can gain admittance. Accordingly, animal husbandry has enabled it to become an important parasite of man and pigs in temperate regions.

Another group has moved to the large intestine and given rise to the oviparous whipworms. This genus is widespread in mammals but is reasonably host specific. Species occur in marsupials, primates, carnivores, rodents, pigs, camels, and ruminants; none has been recorded, however, from other ungulates. The most specialized member of this group is the genus *Trichosomoides* which lives in the urinary bladder of rats. It is really a *Capillaria* in which the male has become parasitic within the vagina or uterus of the female.

There is a great difference in the evolution of the capillarid family of nematodes from that of the oxyurids. Each apparently arose independently and at different times. The capillarids were much older and probably arose from aquatic ancestors. Their thick-shelled egg and long developmental cycle outside of the body were suitable for bottom-feeding aquatic hosts and when these became terrestrial and acquired their first oxyurids, the genus was already well established. It has become extremely widely distributed throughout the vertebrates but is never numerous; its life cycle is against that. This is equally true of the modified forms—except for the viviparous *Trichinella* which in becoming viviparous has apparently avoided host specificity and is restricted in its distribution entirely by the feeding habits of the host. The larger, more specialized whipworms are cosmopolitan in their distribution, and must have originated in the Mesozoic. They may be on the way to becoming hermaphroditic, as in numerous cases females containing and apparently producing fertile eggs have been found in the absence of males, especially in South American hystricomorph rodents. The specialized bladder worm of murids with its super-parasitism is relatively recent.

The oxyurids originated in land animals, possibly from existing forms, more probably independently, and they have remained little changed. Their absence from both groups of ungulates and from carnivores is important. Their intimate life cycle with its short free life, while eminently suitable for arboreal or burrowing animals sleeping in confined spaces, is much less suitable for either carnivores or grazing animals and this probably explains their absence in these groups and their presence in rabbits, rodents, opossums, cobegos, and the reptile-primate group. The viviparous forms were more successful in retaining a wide host range, probably by dispensing with the need for any free life at all. Infections could then develop, like a protozoan or bacterial infection, from ingestion of a few larvae.

A third and entirely different group of nematodes is found in the bursate nematodes, an enormous group which gets its name from a secondary male character—the bursa. This is a membrane-like structure supported on each side of the body by three groups of elongated papillae, the dorsal of which on each side almost invariably fuse together so that the complete structure has two sets of ventral, two of lateral, and one of dorsal rays. There can be no doubt whatever that all bursate nematodes have a common ancestry. Some have developed complicated sucking mouth-capsules to enable them to feed on tissues. Others have retained the more primitive simple mouth. While there is a considerable variety of types, there are three basic groups· the *sclerostomes* of the large intestine, the *hookworms* of the small intestine both with capsules- and the *trichostrongyles* of the stomach and small intestine, without capsules. All have a similar life history involving a free-living, free-feeding larva which hatches from an egg passed in the faeces and which re-enters the body as a contaminant of food; however, some have developed the ability to pierce intact skin, others can enter the body only through mucus membrane or abraded skin, while still others possess this ability only as a potential character. Those which live in the small intestine of carnivores and primates are usually good skin penetrators but they also can enter the body as a contaminant of food. Skin penetration is probably a secondarily developed character.

Now, while these parasites undoubtedly existed before the advent of grass and the development of a cropping as distinct from a browsing method of feeding among the ungulates, there can be little doubt that their enormous radiation must have started about that time—probably in the late Eocene. A ground-feeding habit is extremely desirable for their development while if the host is a browser and feeds on leaves, many larvae must die on the ground. Consequently, the egg production of any individual worm must be large. Once the habit of the host changed, however, many larvae which in earlier eras would have died, could re-enter the body, not only of their own host but of other grazers also. The opportunities for successful mutants to develop were enormously increased. The development of a gregarious habit in the host still further increased these opportunities and consequently they increased in numbers and kinds.

The sclerostomes of the large intestine occur in a large variety of animals but they reach their greatest abundance and variety in elephants, in equines and rhinoceroses, and in Australian marsupials—three extremely old groups of mammals. None occurs in American marsupials. However, there are some extremely interesting species in other animals.

Two species occur in the American tapir, belonging to genera found in elephants and rhinoceroses; there is none, however, in the Indian tapir. One rather isolated genus (*Eucyathostomum*) occurs in South American peccaries, deer, and agoutis, with separate species in each host. Several species occur in the ostrich and rhea. Three species closely related to each other occur in South American tortoises, but no others have been recorded from reptiles.

In Old World artiodactyles the sclerostomes are replaced by the closely related oesophagostomes as parasites of the large intestine.

The presence of fourteen genera of sclerostomes in Australian marsupials and so many in equines, rhinoceroses, and elephants suggests a common and early origin for these groups. Their complete absence in South American marsupials suggests an early divergence of the Australian forms from the main marsupial stem. It is possible to postulate a *double* origin of the marsupials—an early departure of the South American forms in the early Cretaceous from the main *pre-placental* stem, and a later departure of the Australian marsupials (some of which actually do have a primitive placenta) in the later Cretaceous from the *primitive placental* stem.

It has been suggested that the placental mammals descended directly and independently from the pantothers and that the present marsupials evolved independently in the late Mesozoic. The parasitological evidence suggests that while this may well be true for the American marsupials, it is not true for the Australian ones and that these two groups are not only *not* closely related to each other, but may well belong to different orders of mammals.

The presence of sclerostomes in the ostrich and the rhea, alone among the birds, is difficult to explain. The two worms are fairly closely related to each other—in fact some workers have placed them in the same sub-family. However, they are also fairly close to those occurring in equines. It is well known that ostriches, zebra, and gnu habitually to this day congregate together in the veldt, and it is quite possible that the ostrich worm has been thus acquired as a mutant from an equine species. This cannot explain the forms which occur in the rhea, which lives in flocks by itself on the South American plains. Now, there are several species of sclerostomes recorded from South American tortoises—the only forms recorded from reptiles—and one of these has been considered as showing generic relationship with the rhea species (as well as to a sclerostome recorded from *Procavia* in Africa). This view is not correct—the species in the rhea are much more closely related to those in perissodactyles and elephants- but it does suggest a possible, even if improbable, reptilian origin for the rhea species. Tortoises are a relict group and the presence of three sclerostomes in them certainly tends to show the extreme antiquity of these nematodes.

The rhea may have contracted its parasites from the now extinct protoungulates which once roamed South America or it may have contracted them from Africa at the same time as did the ostrich or their ancestors; the fact that, as will be seen, both birds possess an identical tapeworm is in favour of this thesis. The emu and the cassowary have no such sclerostome parasites.

The presence of *Eucyathostomum* in South American deer, peccaries, and agoutis, and of two species in tapirs belonging to genera in elephants and rhinoceroses, is also of considerable interest.

South America has been isolated from North America until the Pleistocene and during that time it developed its own peculiar fauna of cebid monkeys, edentates, opossums, hystricomorph rodents (of which the agouti is one), and the now-extinct protoungulates. The other mammals entered South America from North America when the two continents were joined in the Pleistocene. These other animals are the remaining rodents (squirrels, mice, and so on), the carnivores, the peccaries, the tapirs, the llamas, and a few deer, as well as some now extinct proboscids. The protoungulates although still extant were disappearing at the time of the entry of these strays from the north.

There is no record of any sclerostomes resembling *Eucyothostomum* in artiodactyle ungulates elsewhere than in South America.[3] The fact that there are three species, two in relatively recent immigrants from North America and one in a native rodent, suggests not only a common but a recent origin for all three. The occurrence in the South American tapir of two species of sclerostomes belonging to genera present in African and Indian elephants and rhinoceroses, and the complete absence of any in Indian tapirs may be connected with this. The origin of all may well be some of the now extinct mastodons which not only overran North America but actually penetrated into South America. The fact that African and Indian elephants harbour different species of the same genera of worms shows that their common ancestor must also have been infected with the ancestor of these worms and that this ancestor must be very ancient. The fact that rhinoceroses also harbour species of these genera, shows that the worms have retained some degree of plasticity. The only other origin might be one of the native protoungulates, in which case, however, other sclerostomes might have been expected to occur in South American mammals because of the long association— probably the entire Tertiary era —but none, except those in rheas and tortoises, has been recorded.

---

[3] The only other sclerostomes reported from artiodactyles occur in African wart hogs and are not really members of this group, but probably are aberrant oesophagostomes.

The hookworms are somewhat similar to the sclerostomes but are inhabitants of the small intestine, where they feed on tissue. They are widely distributed in a large variety of vertebrates and appear to be surviving relics of an old, well-distributed group, probably of Miocene origin and possibly from the same stem of bursate nematodes as the sclerostomes.

The Trichostrongyles have been extensively studied in recent years—especially in South America and Australia -and innumerable species have been described. The very simplicity of their morphology makes classification difficult and here, even more than elsewhere, arbitrarily selected characters tend to be its basis. However, it does seem possible to divide them into two main sub-groups- one, probably the more primitive, with a double genital apparatus in the female—the other, with a single genital apparatus. It is possible that the second group is not a homogenous one, the loss of one of the female gonads being not infrequent among the nematodes.

Those with a double genital system are common in ungulates—particularly in ruminants, pigs, and peccaries and in the sciuromorph rodents. One genus (*Molineus*) is common in carnivores in all geographical regions and has also strayed into primates. There are eight or nine genera in bats, two in amphibia, two in reptiles and one in lampreys. One genus occurs in American opossums; three quite different genera in Australian marsupials, while still a fourth occurs in monotremes, with one species in the Tasmanian devil. Nine or ten genera occur in edentates and several in birds; however, one genus is confined to the emu and a second, which occurs in the ostrich, also occurs in the gorilla and a Nigerian rodent. One genus is common in rabbits and a very closely related one in South American hystricomorph rodents—the peculiar group which appeared in some abundance in South America when it was apparently an island, separated from the rest of the world.

The forms with a single genital tube are predominantly parasites of rodents. Some genera are quite cosmopolitan -particularly *Longistriata* with species in all rodent groups, in South American opossums, and cebid monkeys. Most are more restricted. A group of rather closely related genera occurs in the hystricomorph rodents, two in edentates, and one in opossums in South America. Two genera occur in insectivores but show some evidence of a rodent origin. All the others are in holarctic or recently cosmopolitan murid or sciurid rodents and none occurs in Australian marsupials; with a single exception, they are absent from ungulates.

Cestodes. In recent years tapeworms have been the object of considerable study but there is still no general agreement as to their taxonomy and general relationships.

There is little doubt that the cestodes are the most primitive of the parasitic flatworms and possibly even more primitive than the earliest parasitic nematodes. The fact that they are more primitive than the trematodes renders untenable the popular idea that they are really trematodes which have lost their digestive tract. It is more than doubtful if their ancestors ever had a digestive tract and they may well have evolved from free-living saprozoic forms.

Taxonomists classify the cestodes in several groups based mainly on the scolex and the distribution of the genitalia, but there is considerable difference of opinion as to the phylogeny of the groups. Several families occur in elasmobranchs and these are generally regarded as the most primitive species. One family, the Proteocephalidae, occurs in fresh-water fish, amphibia, and reptiles and is usually accepted as representing the stem from which the taenoid cestodes of birds and mammals have evolved.

Among these, the family Anoplocephalidae is regarded as being most closely related to this original stem. Most of its species occur in mammals, but some occur in reptiles and in birds. The family is characterized by the absence of a rostellum and hooks, and by the possession of an egg with three shells—a somewhat archaic character. Only a few life cycles have been worked out in this family but in each case the intermediate host has been found to be a mite.

The most primitive group of the Anoplocephalidae is the subfamily Linstowinae, an assemblage of closely related genera, the points of differentiation being more matters of convenience than phylogeny. Nevertheless, each genus does represent a group of species more closely related to each other than to the species of other genera. The most primitive genus is *Oochristica*. It has a world-wide distribution in reptiles, while in South America it occurs in marsupials, edentates, and monkeys, and in the Old World in insectivores and in mustelid carnivores.

In Australia, it is replaced by the closely related genus *Linstowia*, which parasitizes both monotremes and marsupials. A group of species has developed from this *Linstowia* strain in Australia and has given rise to a number of closely related genera, each a little further away from the prototype.

Probably in Africa, a third group arose in rodents and herbivores, possibly from the same prototype as the Australian group; of these one sub-group occurs in hyracoids, elephants, rhinoceroses, horses and tapirs, one in camels and ruminants, one in rodents, and one in birds. However, one genus of this group —*Thysanosoma* -occurs in ruminants in certain restricted areas in North America and it may have originated there from ancestors carried as parasites of the ancestors of pronghorns.

The genus *Bertiella* which belongs to this family occurs only in primates and cobegos and two very closely related species have been described -one from the New World and one from the Old. The genus probably had an Old World origin, its original morphology being little different from what it is today. It can live in man and although it is not a normal human parasite, it has been recorded sufficiently often to show that the reasons for this are ecological rather than physiological. It is carried by mites and if man still habitually fed on uncooked roots and similar material, *Bertiella* would probably still be a common human parasite.

The great majority of the other taenoid cestodes are avian forms and each order of birds-- even some sub-orders has its own particular tapeworm fauna. This rule is so absolute that in Fuhrmann's opinion any reports which appear to be exceptions to this rule are erroneous. The study of these cestodes shows that a single genus or a single species can be harboured by avian hosts of the same group, even if separated by unpassable barriers. Thus, *Nematoparataenia* is an abnormal mutation preserved by the isolation of its hosts; it has two species—one in the black swan in Australia and one in the mute swan in Sweden. Flamingos in Africa and South America both harbour *Amabila lamelligera*, a very specialized form. Perhaps the most striking example, however, is the occurrence of the genus *Houttuynia* in the African ostrich and the South American rhea. Moreover, the species are the same and the genus occurs in no other birds. The cassowary and the emu have tapeworms belonging to the genus *Raillietina*—a cosmopolitan genus with species in all zoogeographical areas.

The great variety of taenoid cestodes in birds may well be associated with the explosive development of these vertebrates from non-feathered reptiles in the late Mesozoic. If they were parasitized by a relatively labile stock of taenoid cestodes, the change in the biochemistry of their digestive tract would offer opportunities for mutants to obtain a successful habitat, to develop first physiological and subsequently morphological differences. In this way each order of birds would receive its quota of specific cestodes.

The larval stage of the taenoid tapeworms is a tapeworm head without sex organs or segments. It is usually found in an arthropod and it has been suggested that the original hosts of these organisms were the arthropods in which only the scolex now develops. The majority occur in birds— the typical arthropod-eating vertebrates. Some of the tapeworms in mammals still have arthropod vectors—that is the case in the family Anoplocephalidae. Some, however, have other vertebrates as the intermediate host and that appears to be the case with the family Taenidae, the larval stage of which is a comparatively large scolex—sometimes multiple.

In this family, two species occur in carnivorous marsupials in Australia, while one genus occurs in accipitiform birds; various species of *Taenia* occur in carnivora, in man, and in kites, gulls, and penguins, all carnivorous animals. It seems probable that the family has evolved as the result of the habit of eating other vertebrates and it may even have been originally an avian group. It is quite possible that it is the most recent of all the families of tapeworms.

This would explain the occurrence of two members of this family in Australian carnivorous mammals. While the life history of these species is unknown it seems possible that the intermediate host is either a fish or one of the numerous species of placental rodents indigenous to Australia - there are over fifty species of them.

The two human species (*Taenia solium* and *T. saginata*) are probably mutants from canine forms. *T. solium* is still very similar to *T. ovis* of dogs and even yet its adult stage will commence to develop in the intestine of these animals, although it will not reach sexual maturity. *T. saginata* is a more specialized form with a strict host specificity and a more specialized morphology, and it is probably older.

An interesting development in this family is seen in the genus *Echinococcus*, where a considerable reduction in size and, consequently, egg production is accompanied by a corresponding asexual multiplication in its larval stage; this is accompanied by an almost complete loss of larval host specificity.

What is probably the final stage in the evolution of taenoid cestodes in general is shown by several small species which are losing or have already lost the necessity for an intermediate host at all: the immature head (or cysticercoid) develops in a villus of the intestine in the lumen of which it will ultimately reach maturity.

HOST PROBLEMS

Time does not admit of a detailed analysis of the information presented by these various groups of parasites, but there are obviously a number of problems and suggestions of very considerable importance uncovered in even this superficial survey.

Birds. One of the most interesting of these concerns the ratite birds. The ostrich and the rhea are the only birds with sclerostomes—separate but related genera. They harbour an identical species of tapeworm and they have different kinds of trichostrongyle worms from other birds. Moreover, their parasites do not resemble those of the other ratite birds. The parasite evidence suggests most emphatically that they had a common origin in Africa or Africa-South America from a stock different from other birds—as is suggested on other evidence. It also suggests that they are not closely related to the emu and cassowary—or even to the tinamous, as is generally maintained.

Marsupials. The parasitic fauna of South American marsupials and of Australian marsupials, while showing some resemblances, is actually quite different. In Australia, there is a great multiplicity of bursate nematodes, and those forms with mouth capsules are particularly well represented. The tapeworms, while belonging to the same cosmopolitan family as those of the American opossums, are of different genera and species, strongly suggestive of a separate evolution after separation. Oxyurids are virtually absent, those which are present being apparently related to *Passalurus* of rabbits. In South America, the bursate nematodes are rare, tapeworms are more primitive, and the peculiar oxyurid, *Cruzia*, is common.

The evidence is strongly suggestive of an origin of the opossums earlier than and independent from that of the Australian marsupials, with a fauna inherited from these days retained partly by isolation and partly by a continued arboreal habitat. Many of these South American parasites belong to genera shared by edentates (which are survivors of another very primitive group) and some have passed into cebid monkeys.

The great increase in bursate nematodes in Australian marsupials is probably associated (as it is in the ungulates) with the secondary adoption of a terrestrial and a grazing habit. This would infer that they developed after the late Eocene but that their predecessors came to Australia before that time. Their resemblance to the sclerostomes in perissodactyles, rather than to those in artiodactyles, strongly suggests, if not a common origin, at least a common habitat with the primitive perissodactyles. It further suggests that the artiodactyles with their modified oesophagostomes, had a later origin--after the Australian marsupials had separated.

Insectivores and primates. The insectivores have been examined quite superficially for the presence of parasites but such forms as have been reported agree well with the concept of their very generalized character. These include *Plasmodium*, two anoplocephaline cestodes (including the primitive *Oöchristica*), and some trichostrongyles. Perhaps the best evidence of their primitiveness (as it is for hystricomorph rodents) is their ability experimentally to harbour so many parasites of other mammalian groups.

The parasites of the flying lemurs or Cobegos also have been little studied but one specialized oxyurid genus, and two anoplocephaline tapeworms have been recorded from *Galopithecus*. Of these, the tapeworms are of particular interest because one is *Bertiella* of primates, the other *Prototaenia* of Australian marsupials. The systematic position of these creatures is still obscure, but their tapeworm fauna prompts the suggestion that they may be close to that point in the placental stem at which the Australian marsupials diverged from the proto-insectivores. Their parasites show no resemblance to those of bats, which have a number of very specialized forms.

The parasites of monkeys have also been insufficiently studied to make many generalizations possible. Their malarial parasites and *Bertiella* have already been discussed, while the cebid monkeys at least have been shown to be able to act as hosts to a variety of parasites of diverse mammalian groups—testifying to the generalized state of their physiology and consequently its comparatively unspecialized and primitive nature. Apart from the development of intelligence in the more advanced members of the group, the primates in general are really very primitive animals.

The malarial parasites of man have also been discussed in detail in an earlier section. They show a line of descent from his remote ancestors in the Mesozoic. His tapeworms, he has acquired from a number of sources. *Bertiella* he was infected with before he left the prehuman stem and he could still have it if he included the suitable mites in his diet; his changed mode of living has made it rare. His two taenias he acquired in the days of human pre-history when he adopted the dog and domesticated the ox and the pig; *T. solium* is probably younger than *T. saginata*. The fish tapeworm (*Diphyllobothrium*) is probably the most recent of his regular tapeworms and may well be, as some authorities claim, a neotenic larva, the ancestors of which reached sexual maturity in a fish—possibly in Pliocene times—then later became adapted to carnivores and finally to man. *Hymenolepis* he has taken, or is taking over from domestic rodents, while the others are accidental visitors from carnivores and rodents.

His roundworms were probably the result of several evolutionary lines. His pinworms were familial parasites inherited from pre-insectivore days. One hookworm came from carnivores, the other from ungulates. While some of his other nematodes are obviously inherited from ancestors, many are quite accidentally acquired from other mammals.

The ungulates. The ungulates are not a natural group but include all the larger placental herbivores with teeth arranged for grinding and cropping and limbs arranged for rapid locomotion—although there are obvious exceptions to this. Living representatives are usually classed in three groups—the sub-ungulates (the conies, elephants, and sea-cows), the odd-toed perissodactyles (horses, tapirs, and rhinoceroses) and the even-toed artiodactyles (the bovines, deer, giraffes, camels, pigs, and hippopotami). In addition there is an extinct group of some importance—the protoungulates of South America.

Ungulates were unknown in the Mesozoic era, but by the end of the Palaeocene in the Tertiary, four archaic groups of protoungulates were in existence. They were rare outside of South America, but in that continent, which is believed to have been isolated during most of the Cenozoic, the group expanded enormously into a great variety of forms, ranging in size from an elephant to a rat, and it was still in existence when South and North America were united in Pliocene times. However, they were then on their road to extinction and the wave of carnivores which entered from the north completed their disappearance. With the carnivores also came a very few true ungulates, llamas, peccaries, the tapir, and some deer. Later, of course, man introduced cattle, sheep, pigs, and horses to complicate the parasite picture.

The sub-ungulates originated in Africa—the conies are still found only there, although the proboscids spread all over the world and even reached South America in the late Cenozoic; today they exist only in tropical Africa and Asia as two quite distinct species—with a very similar parasitic fauna.

The perissodactyles may have originated in a stem in the late Palaeocene but the sub-groups quickly separated and the living groups, the horses, the tapirs, and the rhinoceroses, have been separated from the early Eocene at least. Their modern representatives are few in number and the group is heading towards extinction.

The main growth of existing ungulates is the Artiodactyles, but it is only in post-Eocene times that they have become dominant and some, like the Bovidae, did not develop till the Miocene. There are two main groups—the ruminants and the pigs. Of the ruminants, the camels and llamas and the tragulids—both diminishing groups—go back to the Eocene; the other are all Miocene. The hippopotami may be traceable to a group, now extinct, which originated in the Eocene, while the pigs and peccaries appeared in the late Eocene but separated in the Oligocene.

The diversity of types of sclerostomes shows that these worms had a very ancient origin - possibly (as their presence in tortoises suggests) a reptilian origin. The presence of closely related species in sub-ungulates and in perissodactyles suggests a common origin for these two groups, earlier than that of the artiodactyles with their modified oesophagostomes. The tapeworm fauna agrees with this hypothesis. Their possible relationship with the Australian marsupials has already been noticed.

Edentates. The parasites of the surviving edentates show certain resemblances to those of other South American mammals. The tapeworm, *Oöchristica*, is cosmopolitan and is common in opossums and in reptiles, as well as in cebid monkeys and lemurs, in hedgehogs, and mustelid carnivores. Several species of trichostrongyles have been recorded from them, in genera peculiar to them, but showing an inter-edentate relationship. Of these genera, one is closely related to a genus in opossums, while others are related to two ruminant genera of probable African origin.

Glires. The rodents and the rabbits are regarded by mammalogists as not closely related to each other. However, their parasites are—particularly those of the hystricomorph rodents—the agoutis, chinchillas, nutria, and their relations. This group is predominantly a South American one where, except for a few immigrants from North America, other rodents are absent. The hystricomorph rodents, except for such immigrants to North America in the Pleistocene as the American porcupines, occur only in South America with a minor focus of porcupines and cane rats in West Africa. (The porcupines which occur in Eurasia are not closely related to them.) Their great development was in South America in the Oligocene and they were absent in the previous epoch when South America is believed to have been isolated from all other lands. While there is a general belief that they originated in Africa, there are no old North American fossils.

The rabbits, which were apparently present in Holarctica in the late Eocene and Oligocene and may well have originated in Asia and in the Palaeocene, have parasites quite close to those of the South American rodents.

The parasitological evidence suggests a definite post-Mesozoic connection between South America and Africa, possibly in the Palaeocene. It does not postulate any connection between Australia and South America after the origin of the mammals, although it does suggest one between Australia and Africa shortly after this time.

This very rapid and very superficial survey of some of the potential implications of parasitology is necessarily incomplete and inconclusive. The available data, if nothing else, impose strict limitations on the suggestions it may indicate. Better and more accurate morphological studies would strengthen the force of these suggestions and enable more definite conclusions to be drawn. Especial attention should be paid to the apparent exceptions because they are the real clues, the real tests to the rules—and this requires a much greater enthusiasm for parasites than has existed in the past. However, it does help to direct attention to the value of a true taxonomy and to stress, not only the importance of integration of all branches of biological science, but the need for synthesis as well as analysis in that integration. It helps to draw attention to the fact that parasites are part of the hosts' environment and may evolve with it and that a better understanding of these very intimate associations may help to untangle the complicated relationship of their hosts.

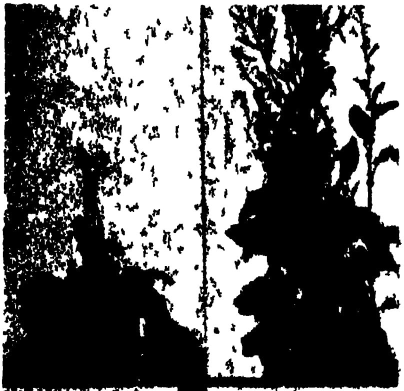
TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIV : SERIES III : JUNE, 1950
SECTION FIVE

A Tetraploid Form of Annual Rape Induced by Colchicine

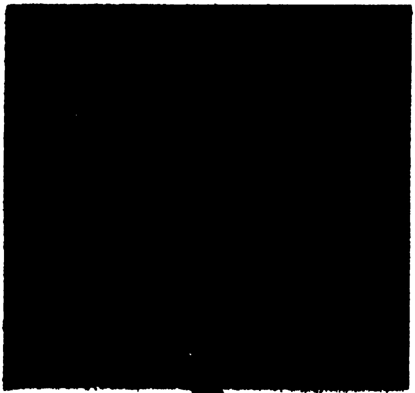
By J. M. ARMSTRONG
Presented by N. H. GRACE, F.R.S.C.

ANNUAL rape, known as German or Polish, *Brassica Rapa annua oleifera* Metzg., has long been cultivated in Europe as a source of edible oil. Together with Argentine rape, *Brassica napus oleifera annua* Metzg., these crops were introduced to Canada in 1942 with a view to overcoming the shortage in vegetable oils. They proved well suited to certain areas in Western Canada and by 1948 the acreage had increased to 80,000 with a total yield of 64 million pounds. There was a strong demand for rape oil during the Second World War as a marine lubricant and following the war the European demand for rape oil for edible purposes was steady. But in the last two years the European market demands have declined and rape oil has not yet established a place on this continent in competition with other vegetable oils. Consequently the acreage of this crop in Western Canada has been drastically reduced in spite of the obvious desirability of suitable substitute crops for a portion of the wheat acreage. Rapeseed oil has been used widely as a source of margarine and cooking fats (Grace and Zuckerman, 1947). Appreciation of the food value of rapeseed oil could lead to its attaining an established place in Canadian agriculture.

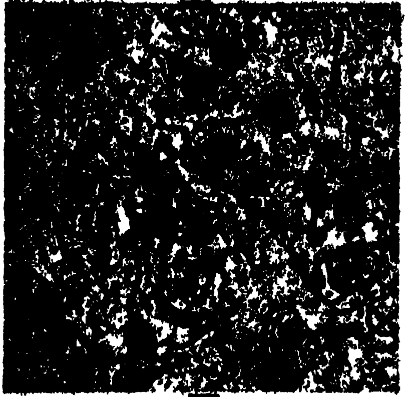
German rape is a diploid species (2n = 20) while Argentine is an allopolyploid (2n = 38). The latter has presumably arisen by the hybridization of species with 18 and 20 somatic chromosomes followed by chromosome doubling. The two rape species stand in the same relationship with each other as the two *Brassica* species grown as field roots, the swede (2n = 38), *Brassica napus* (L.) var. *napobrassica* (L.) Reichb., and the turnip (2n = 20), *B. campestris* (L.) var. *rapa* (L.) Hartm. Due to the lower chromosome number German rape has proven more favourable material for inducing polyploidy than Argentine rape. The latter yields only dwarfish, highly sterile plants when the chromosome number is doubled (Plate I, Fig. 1). Similar results were obtained by Armstrong (1942) in attempts to double the chromosome number in the swede species.



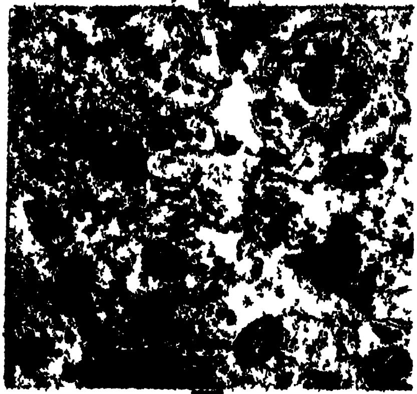
1



2



3



4

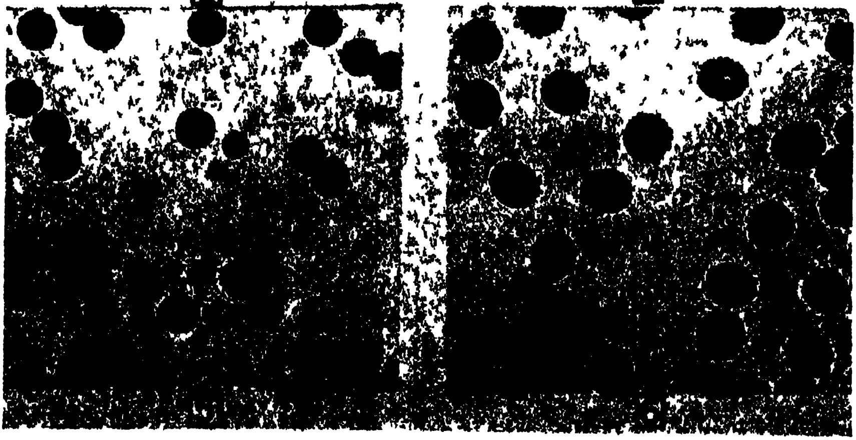


PLATE I

The present paper reports the production of tetraploid strains of German rape by means of colchicine treatments. The comparative yields, meiotic behaviour, and chemical analyses of these strains are also reported.

LITERATURE REVIEW

Since the discovery of the use of colchicine as a means of chromosome doubling by Dustan (1934), the possibilities of obtaining improved varieties by this means have been widely explored in many economic crop species. In forage crops alone the production of tetraploids has been reported for *Medicago sativa* (Julen, 1944), *Trifolium pratense* (Levan, 1940), *Trifolium repens* (Atwood, 1944), *Trifolium hydridum* (Levan, 1940), *Melilotus alba* (Johnson and Sass, 1942), *Zea mays* (Randolph, 1935), *Lolium perenne* (Myers, 1945), *Lolium multiflorum* (Shalygin, 1941), *Sorghum vulgare* var. *sudanese* (Salomon, 1944), *Panicum miliaceum* (Arenkova, 1940), *Stipa lepida* (Stebbins, 1942), *Dactylis aschersoniana* and *Festuca elatior* (Myers and Hill, 1947) and *Soja max* (Porter and Weiss, 1948). Some of the objectives sought were increased yield and vigour, greater hardiness and longevity, and a favourable modification of the chemical constitution. While in several instances the autopolyploids have been markedly more vigorous than the corresponding diploids they have been generally characterized by reduced fertility.

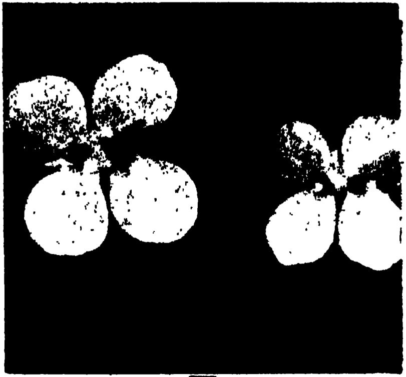
Darlington (1937) explains this reduced fertility as being due to the formation of one to several multivalent chromosome associations which divide irregularly and produce gametes with aneuploid chromosome numbers. Myers (1945) considers that the quadrivalent frequency as a cause of irregularity must be considered in conjunction with chiasma formation, a higher chiasma frequency causing less irregularity than a lower one. Randolph (1941) concluded from a study of tetraploid maize that lowered fertility may be of a physiological nature although under genetic control. Clausen *et al.* (1945) reported an instance of this type of sterility in the amphidiploid, *Layia penta-*

FIGURE 1. —Double chromosome form, left, and normal Argentine rape, right. This species did not respond favourably to induced doubling of the chromosomes.

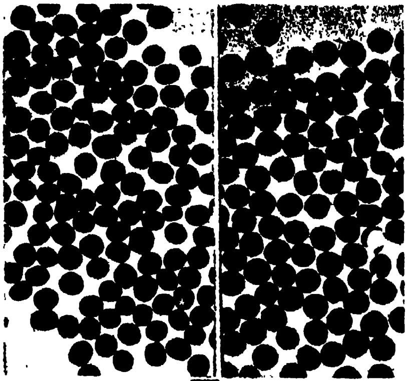
FIGURE 2. —Comparison of leaves of diploid, above, and tetraploid, below. Besides being larger than the diploid, the tetraploid has a different shape index and more strongly indented margins.

FIGURES 3 and 4.—Comparison of stomata in diploid, 3, and tetraploid, 4. The lower epidermis of the leaf was stripped and fixed in 95 per cent alcohol to which a few drops of light green were added.

FIGURES 5 and 6.—Comparison of pollen size in diploid, 5, and tetraploid, 6. The diameter of the diploid was 25 microns and of the tetraploid 32 microns.



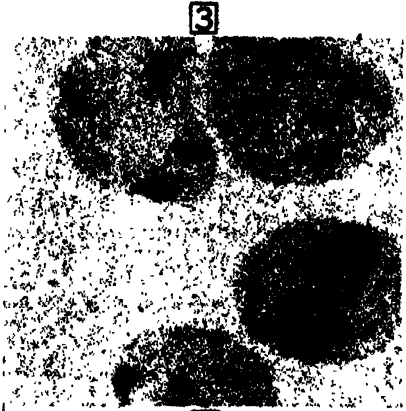
1



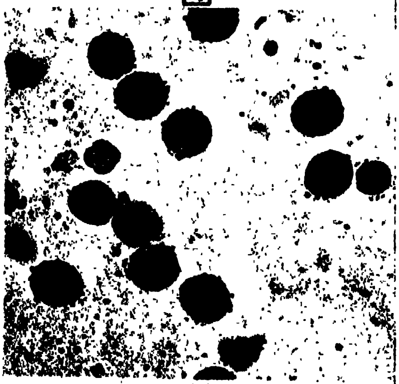
2

3

4



5



6

PLATE II

glossa, where chromosome pairing was fairly regular at first metaphase but was extremely irregular in the remaining stages of meiosis, owing apparently to basic disturbances in the genetic balance.

Several cases have been reported where the diploid balance in chemical constituents has been modified as a result of chromosome doubling. Sullivan and Myers (1939) found from an analysis of green fodder in *Lolium perenne* that the tetraploids were higher than the diploids in reducing sugars, sucrose, total sugars, and the proportion of dry matter soluble in 80 per cent alcohol. Porter and Weiss (1948) working with soybeans found that the tetraploids were substantially higher in protein, materially lower in oil content, and slightly lower in iodine number than the diploids. On the other hand Julen (1944) reported that in alfalfa diploids, triploids and tetraploids did not differ materially in chemical constitution. Peto and Boyes (1940), Armstrong (1942), and Abegg *et al.* (1946) found that in sugar beets doubling the chromosome number did not result in an increase in sugar yield per acre over the original diploids. For many of the induced polyploids chemical analyses have not been reported.

EXPERIMENTAL RESULTS

Production of tetraploids. The production of the tetraploid in German rape offered no special problem. The seed was first presoaked 24 hours to initiate germination. It was then treated with a .2 per cent aqueous solution of colchicine, first under reduced pressure for one-half hour followed by six hours' soaking. The seed was then washed and planted in trays in the greenhouse. The affected seedlings, as judged by thickened stems and leaves, were then transplanted to small pots. In late June 1947, 168 seedlings and 25 untreated checks were transplanted to a small field nursery. Upon flowering the pollen size was examined microscopically. In the tetraploids the pollen was about 1 1/3 times the diameter of the normal diploid (Plate I, Figs. 4 and 5). This afforded a relatively simple basis for separation. Seventeen plants were selected and selfed seed obtained by bagging un-

---

FIGURE 1. -Comparison of flower size in diploid, left, and tetraploid, right.

FIGURE 2. -Comparison of seed of diploid, left, and tetraploid, right Besides being somewhat larger the tetraploid possesses more uniformity.

FIGURE 3.—Metaphase I of pollen mother cell of tetraploid. The figure contains 8 quadrivalents and 4 bivalents.

FIGURE 4. -Late anaphase I in pollen mother cells in tetraploid. All 5 cells are free from any irregularities.

FIGURE 5.—Metaphase II and telaphase II in tetraploid.

FIGURE 6.—Pollen of plant 17-3 showing two abnormally small grains.

TABLE I

RESULTS OF CROSSES BETWEEN TETRAPLOID PLANTS OF GERMAN RAPE

Code number of female parent	*Number of crosses*	*Total number of pods*	*Total number of seeds*	*Average seeds per pod*	*Seed weight per 1,000 gm.*
17	7	41	366	8.9	3.56
38	3	33	324	9.8	3.26
41	4	29	349	12.1	3.03
43	3	27	293	11.0	2.93
46	2	9	59	6.2	3.73
50	4	35	392	11.2	3.09
63	3	32	373	11.7	3.31
66	4	44	450	10.3	3.41
67	7	63	715	11.3	3.61
68	5	50	365	7.3	2.50
Total	42	363	3,686	11.6	3.19
Diploid check					2.04

opened inflorescences. Small progenies of these plants were grown in the greenhouse in the winter of 1947-8. The condition of tetraploidy was verified by chromosome counts made from root-tip smears and examination at meiosis. The tetraploids were then intercrossed extensively.

Table I summarizes the results from this intercrossing. The racemes chosen for crossing were trimmed down to twelve flowers and emasculated in the bud stage. When the buds were fully open and the stigma receptive they were cross-pollinated.

In all, 42 crosses were made and 3,686 seeds were obtained for an average of 11.6 seeds per pod. The pollen appeared normal and abundant and no marked sterility was encountered except in the cases where plants 46 and 68 were used as females, in which cases 6.2 and 7.3 seeds per pod were obtained. It is worth noting that crosses using plant 46 produced the largest seeds. The weights for all crosses averaged 3.19 grams per 1,000 seeds, about 50 per cent larger than the diploid (Plate II, Fig. 2).

Other differences in the appearance of diploid and tetraploid plants are apparently in the leaves and flowers. The tetraploid leaves are broader in proportion to length than in the diploid and have more strongly indented margins (Plate I, Fig. 2). The stomata on tetra-

TABLE II

STRAIN TESTS FOR TETRAPLOID GERMAN RAPE, 1948

Strain number	*Yield of seed from 10 ft. row*	*Weight per 1,000 seed*	*Strain number*	*Yield of seed from 10 ft. row*	*Weight per 1,000 seed*
	gm.	gm.		gm.	gm
5	40.8	3.02	4	22.5	2.36
15	34.7	2.66	34	15.1	2.39
3	33.9	2.50	2	14.1	2.48
1	32.3	2.69	7	13.9	2.45
11	24.9	2.14	6	13.3	2.23
13	23.6	2.43	9	13.3	2.25
14	22.9	2.65	38	8.5	2.26
Least significant difference at at 5% point	12.9	.39		12.9	.39

ploid leaves are distinctly larger (Plate I, Figs. 3, 4). This is a fairly reliable plant character in diagnosing induced tetraploidy. The tetraploid and diploid flowers show a striking difference in size (Plate II, Fig. 1). As previously mentioned the pollen is also larger in the tetraploids.

Comparative yield of tetraploid strains. A field nursery of the first generation tetraploid crosses was grown in 1948. To make the most of the small seed supply the plants were started in pots in May and then transplanted to the field in June and given a four-inch spacing in rows 10 feet long. In fourteen of the crosses there were sufficient plants for three rows of each and these were replicated in a random manner so that the yield data could be analysed statistically.

The seed yield and weight per 1,000 seeds for this test are given in Table II. The analyses of variance showed that differences in strains were significant for both seed yield and seed weight. Four lines or crosses above the average in yield were 1, 3, 5, and 15. These lines were above average for seed weight also. It was not considered advisable to include diploid rows in this nursery for purposes of comparison owing to the danger of cross-pollination which would result in triploid seed.

A further test was carried out in 1949 using 21 of the most promising strains grown in 1948. If a certain amount of crossing took place

TABLE III

STRAIN TESTS FOR TETRAPLOID GERMAN RAPE, 1949

Strain number	*Yield of seed lb. per A.*	*Weight per 1,000 seed* gm.	*Strain number*	*Yield of seed lb. per A.*	*Weight per 1,000 seed* gm.
15	842.7	2.78	1	610.2	2.56
17	789.6	2.65	10	595.9	2.61
3	745.5	2.86	24	581.1	2.72
27	733.0	2.96	29	554.3	2.53
31	705.6	2.57	30	549.7	2.64
13	680.3	2.61	12	539.0	2.57
5	677.5	3.01	32	513.4	2.64
33	668.6	2.76	18	509.8	2.73
35	652.6	2.72	26	472.8	2.69
28	615.1	2.62			
11	613.3	2.38			
25	638.3	2.80			
Least significant difference at 5% point	159.8	.15		159.8	.15

between strains in 1948, the 1949 test may be regarded in the nature of a polycross strain test. This was altogether likely as the honey bees regard the rape as an excellent pollen source and collect very assiduously while the bloom lasts.

Table III gives the results of the 1949 test. Each strain was replicated five times in seeded rows nine feet long. The yields are expressed on the basis of pounds per acre. The nursery was seeded on May 31, and harvested August 19, the growth period being 80 days. Although the 1949 season was not a favourable one, the plants did not suffer excessively from drought and the yields may be regarded as good. The analyses of variance showed significant differences between strains both in regard to yield and seed weight. The five best strains were significantly better than the five poorest. The correlation between yield and seed weight was + .366 which is just on the border line of being significant at the 5 per cent point (19: 1). Since yield depends on several factors it is not surprising that only a low correlation exists between yield and one of its contributing factors.

Comparing the yield of strains for the two years, it will be seen that strain 15 which was second highest the first year is highest the second year. Strain 3 remains in the third position both years. Strain

5, highest the first year, has dropped to the seventh position the second year.

These tests prove a point of some importance to the plant breeder. Since there are significant differences between these tetraploid strains, selection either of superior plants, crosses or strains should be quite effective. A new tetraploid species should not be condemned, unless or until one has the best possible expression upon which to base an estimate.

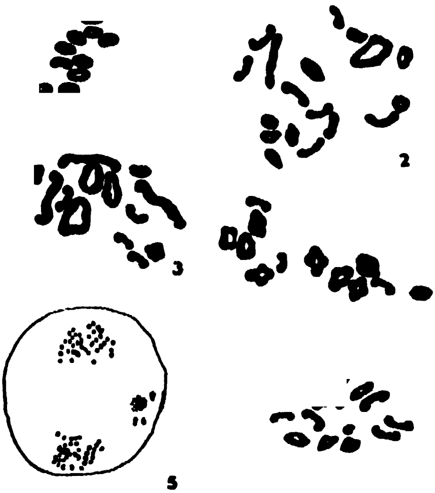


PLATE III

FIGURE 1.—Metaphase I of diploid German rape showing 10 ring bivalents.

FIGURES 2-4. –Typical metaphase I figures of tetraploid German rape. FIG. 2.—This cell has 2 quadrivalents and 16 bivalents. FIG. 3. This cell has 6 quadrivalents and 8 bivalents. FIG. 4. This cell has 8 quadrivalents and 4 bivalents and is identical with that of Plate II, Fig. 3.

FIGURE. 5.—-Early anaphase II of plant 17-3. The two univalents have divided but would reach the poles too late to be included in the nuclei.

FIGURE 6.—Metaphase I of plant 17 –3 showing 4 quadrivalents, 11 bivalents and 2 univalents. Drawn with the aid of camera lucida and reproduced at a magnification of × 2700.

Meiosis. A group of third generation plants were grown in the greenhouse in 1949-50 and meiotic studies were carried out on them. The racemes were at the right stage for fixation when they were just emerged from the leaf axils with florets about one to two mm. long. This microsporocyte material was killed and fixed in acetic-absolute (1:3) and stored in the fixing solution for three weeks in a refrigerator. All data were collected and drawings made from aceto-carmine smear preparations. These were made semi-permanent by sealing with a

TABLE IV

COMPARATIVE DATA ON BEHAVIOUR OF DIPLOID AND TETRAPLOID GERMAN RAPE FROM FIRST METAPHASE TO MATURE POLLEN

Plant material	*Chromosome associations at first metaphase* I	II	III	IV	*Range of* IV	*Irregularities* %	*Normal pollen* %
2x check		10.00					95.4
4x plants							
15 -1		5.00		7.50	5-9	5.0	97.0
26 -1	.11	14.24	.11	2.77	1-7	1.6	98.0
-2		13.00		3.50	1-6	1.2	80.4
—4	.06	8.90	.06	5.50	2-8	6.8	93.4
27—3	.10	8.80	.10	5.50	1-9	4.8	92.7
31--2		10.20		4.80	3-7	3.2	91.5
-4		7.20		6.40	4-10	4.3	93.7
32—3		4.00		8.00	2-8	1.2	95.3
Ave of 4x	.03	8.92	.03	5.51	1-10	3.9	94.0

1:1:1: mixture of aceto-carmine, corn syrup, and Certo. Ten to twenty microsporocytes were analysed at metaphase I for each plant. Additional observations were made up to the pollen tetrad stage and a count of the per cent of normal pollen was made at the time of anther dehiscence.

Table IV summarizes the cytological data collected from a check diploid plant and eight tetraploids picked from various lines. An additional tetraploid plant, 17-3, which contained marked irregularities is not included in this table but will be referred to later. In the diploid plant 10 bivalents were regularly formed at metaphase I. These were mostly of the closed ring type and were comparatively small and well contracted (Plate III, Fig. 1). The terminalization of the chiasmata was fairly complete. Disjunction at anaphase was normal and the chromosomes did not lose their identity at the interphase but passed directly into the second division. In a single anther stages from metaphase I to telaphase II could be observed, indicating the rapidity of the meiotic process. The two lower tetradynamous anthers were slightly behind the four longer anthers in development.

In the tetraploid plants each microsporocyte at metaphase I was composed of an assortment of bivalents and quadrivalents. An occasional cell showed the presence of a trivalent and univalent. No higher multiple associations than quadrivalents were observed. The average quadrivalent frequency per cell ranged from 2.77 for plant 26-1 to 8.00 for plant 32-3. Since this average for plant 26-1 was determined from cells that ranged from one to seven quadrivalents it is doubtful if differences in the quadrivalent frequencies in the various plants can be considered as significant. The grand average for all tetraploid plants was 5.51 or 55 per cent of the possible total. This agrees closely with the figure obtained in tetraploid *Lolium perenne* by Myers (1945) where 56.6 per cent of the chromosomes at metaphase I were in quadrivalent associations. It is much higher than that found in the autotetraploid of *Plantago maritima* L. in which Earnshaw (1942) found the quadrivalent frequency never exceeded two per cell.

The quadrivalent configuration was usually a closed ring type and more infrequently an end-to-end chain. The greater frequency of rings over chains may be taken to indicate high initial chiasma frequency at pachytene. The difference in the degree on chiasma terminalization in the same cell between bivalent and quadrivalent appears significant as shown in Plate III, Fig. 4 and Plate II, Fig. 3. The four bivalents have terminalized chiasmata while five of the eight quadrivalents have distinct interstitial chiasmata which frequently give the configuration the form of a cross. This difference in complete terminalization between bivalent and quadrivalent may be due to the difference in the complexity of the two types of chromosome configurations.

The occurrence of trivalents and univalents was noted only infrequently. In the 120 cells studied they were observed four times on 3.3 per cent. Their presence causes two types of irregularities. The trivalent will give a two to one division at anaphase leading to two 19 chromosome gametes in the tetrad. The two members of the univalent which lag and divide seldom reach the poles of the cell before the onset of metaphase II by the main groups of chromosomes (Plate III, Fig. 5). The inclusion of the univalents in the nuclei of the tetrad appears to be purely fortuitous.

At anaphase I the quadrivalents appeared to disjoin regularly with alternate chromosomes in the ring going to opposite poles. The separation seemed to be uniform and rapid as is apparent in Plate II, Fig. 4 where a number of adjoining anaphases were photographed. The unequal disjunction at anaphase I of the chromosomes in a quadrivalent has been reported by several investigators and is considered to be the principal cause of instability and low fertility in autopolyploids. Kostoff (1940) concluded that a species with short chromosomes characterized by a low chiasma frequency stood the best chance of forming stable autopolyploids since multivalents would be fewer.

Further observations on meiosis concerned the frequency of univalents from anaphase I to telephase II for each plant. Approximately 200 cells of each were examined and, as seen from Table IV, the frequency ranged from 1.2 to 6.8 with an average of 3.9, which approximates that observed at metaphase I. Table IV also gives the percentage of normal pollen observed at the time of anther dehiscence. This was in every case in excess of 90 per cent. The abnormal pollen consisted of a small per cent of micro-nuclei and other grains which were about one-half the size of the normal grains and were deficient in cytoplasm. As the latter type were also observed in the pollen of diploids where the regularity of meiosis was unquestionably good they may be due to physiological disturbances. Another group of 8 diploid and 24 tetraploid plants studied for pollen normalcy at anther dehiscence were distributed as follows:

	Per cent of normal pollen					Ave
	80-84	85-88	89-92	93-96	97-100	
2x plants			1	3	1	96.2
4x plants	1	1	2	12	8	94.1

It may be seen that the averages for the two types vary only slightly in favour of the diploid group.

One plant, 17-3, which was studied at meiosis is not included in Table IV because of the rather puzzling nature of the chromosome irregularity. Although the chromosome number was ascertained with certainty to be 40 and in general this plant showed the same pattern of bivalent and quadrivalent configurations as the other tetraploid plants, it had a relatively high frequency of cells at metaphase I and subsequent meiotic stages where one or two univalents were present (Plate III, Fig. 6). It is postulated that one or both of these univalents were prevented from pairing regularly at pachytene by small segmental inversions. An examination of 100 pollen tetrads showed 28 with 2 to 4 chromosomes outside the nuclei. At least one-half of the gametes from such tetrads would contain aneuploid chromosome numbers. In the mature pollen of this plant there were many micronuclei and a comparatively low percentage for normal pollen of 82.8 per cent (Plate II, Fig. 6). This plant from strain 17 cannot be considered as typical of the strain since in Table III it is seen to be the second highest yielder. It does show that even in autotetraploids characterized by good meiotic regularity and fertility occasional irregular types will be produced.

TABLE V

PHYSICAL AND CHEMICAL CHARACTERISTICS OF RAPE OIL

Sample number of tetraploid lines	*Per cent oil extracted*	*Refractive index*	*Free fatty acid*	*Saponification number*	*Iodine value*	*Percentage of acid*	
	Dry wt. basis	at 25°C.	% as oleic	mgm. KOH/gm. 1	ctgm./gm.	Linoleic	Linolenic
1	31.9	1.4694	0.79	182.2	106.7	19.2	7.8
2	23.5	1.4701	2.31	183.6	108.0	20.5	7.1
3	29.8	1.4700	1.34	184.2	107.6	19.7	7.4
4	30.0	1.4699	1.52	177.0	104.3	15.9	6.9
5	31.8	1.4698	0.66	179.5	103.7	18.4	8.1
6	32.0	1.4706	2.20	181.7	105.7	19.2	4.3
7	34.2	1.4700	1.66	178.5	106.9	17.8	8.1
9	25.2	1.4709	2.41	178.8	108.2	22.1	7.7
11	29.5	1.4705	1.51	186.0	104.9	20.3	7.6
13	30.2	1.4692	1.63	183.5	104.3	19.3	7.3
14	25.3	1.4708	1.46	180.9	105.8	16.6	6.6
15	30.3	1.4703	1.18	183.0	107.3	16.3	7.4
34	26.8	1.4699	2.45	181.0	108.0	18.1	7.1
Ave.	29.3	1.4701	1.62	181.5	106.3	18.7	7.2
German diploid 1	38.0	1.4700	1.35	177.4	107.2	12.9	7.2
2					104.4	15.5	7.6
3						17.7	7.1
Argentine	35.8	1.4690	0.93	176.2	96.6	11.7	6.2

Chemical composition. From the 1948 nursery material enough seed was available from 13 tetraploid lines to carry out chemical analyses, although of necessity the samples were limited to about 75 grams. Three lots of diploid German and one lot of Argentine rape seed were analysed also as checks. These analyses were very kindly carried out by Dr. N. Grace of the National Research Council.

The results of these determinations are given in Table V. Compared to such an oil-seed crop as soybeans, rape seed is a high yielder of oil ranging from 30 to 40 per cent (Lewkowitsch and Warburton, 1921). The percentages obtained from these samples were 38 for the German rape and 35 8 for the Argentine. The German rape oil has a refractive index of 1.4700 at 25°C.; contained 1.35 per cent of free fatty acids such as oleic; had the saponification number of 177.4 (the rape oils being characterized as the lowest of all vegetable oils in this regard). It has a low iodine number being 106.3 for the first sample. The percentages of the two acids determined, linoleic and linolenic, were 12.9 and 7.2 respectively. The comparative values for Argentine rape oil may also be noted from the table and are seen to differ from the German diploid in several respects.

To obtain an idea of the general effect of chromosome doubling on the chemical composition of German rape oil the mean values of the tetraploid strains for the various characteristics can be studied. It may be seen that for refractive index, iodine value and per cent linolenic acid they differ very slightly from the diploid. But for per cent oil extracted, saponification number, percentages of free fatty acids and linoleic acids, the differences are more marked.

TABLE VI

STATISTICS ON TETRAPLOID STRAINS

Characteristic	*Coefficient of variability*
Per cent oil extracted	10.34
Refractive index	.69
Per cent free fatty acid	10.93
Saponification number	1 37
Iodine value	1 43
Per cent linoleic acid	9.09
Per cent linolenic acid	12.97

Another point of interest is the degree of variability existing in the tetraploid strains. This can of course be calculated by their standard deviations but for purposes of comparison the best statistic is the coefficient of variability. The C. V. values are given in Table VI. The strains have low variability for refractive index, saponification number, and iodine number, while variability for the other characteristics is fairly high. From the plant breeder's point of view the latter condition is more desirable as it gives greater scope for selection. It is apparent from Table V that selection would not be effective for such characters as refractive index and iodine value. No amount of selection could conceivably raise the iodine number of a strain to the point where it would be considered a good drying oil. In regard to the linoleic acid content where a certain per cent is desirable, that per cent in either diploid on tetraploid is already ample. A high content of linolenic acid is undesirable for food uses and in this case selection would be possible in the direction of low content While the average of all tetraploid strains for per cent oil extracted is significantly lower than the diploid, some strains such as numbers 6 and 7 are high enough to warrant selection even if further hybridization had to be resorted to with strains 3, 5 or 15 to combine the best expressions of yield and oil content.

DISCUSSION

With the discovery in 1937 of the colchicine method of producing polyploids experimentally, there was a general feeling of optimism among plant breeders that polyploid forms of great practical importance would soon be produced. But after ten to twelve years of experimental data have been accumulated and the findings analysed, this optimism has not been entirely justified. Double-chromosome forms have been produced with most of the important economic crop species and at least preliminary tests carried out so that one can draw conclusions on a fairly wide basis. The generalization can be made that in only very few cases have new autopolyploids proven superior to the forms from which they were derived. At best the production of new polyploids must be regarded as only the first step in a lengthy breeding programme.

We now know there are certain factors which determine the possibilities and limitations of this kind of breeding work. Firstly, each species has an optimum chromosome number at which it shows maximum development. Above or below this level the viability is lowered and if carried far enough a lethal condition is reached. Unfortunately for plant breeders most plant species have gravitated during the course of their evolution to their optimal chromosome number and their natural response to experimental polyploidy is a decreased growth rate.

Secondly, induced autopolyploids appear to have unfavourable properties in common. Their lower fertility appears to be characteristic and makes the breeding work of autopolyploid cereals, oil-seed plants, and other species grown for seed production very difficult and uncertain. Where the economic gain is in greater vegetative development, utilizing the so-called "gigas" characters there may be a satisfactory compensation for lower fertility.

The cause of the lower fertility in autopolyploids is tied up with chromosome behaviour at meiosis where each chromosome has four possible pairing arrangements as univalent, bivalent, trivalent, or quadrivalent. The first and third arrangements are associated and are an obvious cause of meiotic irregularity. The frequency of quadrivalents is not in itself a function of chromosome irregularity unless it is associated with faulty disjunction at anaphase. In the case reported here quadrivalent frequency was relatively high, due presumably to a high initial chiasma frequency in the prophase. Disjunction at anaphase I was quite regular leading to a high proportion of good pollen. The fertility in the tetraploid compares favourably with the diploid and seed yields are somewhat higher in the tetraploids possibly due to the larger seed.

In producing new tetraploids the possibility of an advantageous modification of the chemical constitution must not be overlooked. That such occurs is shown by examples in the literature previously cited. In the present investigation not only did the tetraploid strains differ as a whole in several chemical characteristics but variations between strains were of sufficient magnitude to make future selection feasible.

SUMMARY

1. Tetraploid strains of annual rape were produced excelling the diploid in yield and seed size.

2. The need for selection within tetraploid strains was indicated by significant differences in yield and seed weight.

3. Meiotic studies showed that the tetraploid strains suffered no impairment in fertility due to chromosome irregularities.

4. Induced doubling produced significant chemical effects and the variability of the strains in this respect showed that selection would be effective here also.

ACKNOWLEDGMENTS

The writer wishes to acknowledge his indebtedness to Dr. N. H. Grace, National Research Council, for making the chemical analyses,

and to Dr. T. M. Stevenson, Dominion Agrostologist, for kindly supervision throughout the course of these studies.

REFERENCES

Abegg, F. A., Stewa[illegible] D. and Coons, G. H. (1946). Further studies of sugar beet autotetraploids. Proc. Amer. Soc. Sugar Beet Technologists.

Abenkova, D. N. (1940). Polyploid race in millet, *Panicum miliaceum* L. Comp. Rend. Acad. Sci., **29**: 332-51.

Armstrong, J. M. (1942). Production and value of polyploid field roots. Sci. Agr., **22**: 787-98.

Atwood, S. S. (1944). Colchicine in[illegible] polyploids in white clover. J. A. S. A., **36**: 173-4.

Clausen, J., Keck, D. D., and Hiesey, W. M. (1945). Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. Carn. Inst. Wash. Pub. 564.

Darlington, C. D. (1937). Recent advances in cytology. Blakeston, Philadelphia.

Dustan, A. P. (1934). Action de la colchicine sur le sarcome greffé Crocker, de la souris. Bull. Acad. Roy. Med. Belg., **14**: 487-8.

Earnshaw, F. (1942). Experimental taxonomy. V. Cytological studies in sea plantains allies to *Plantago maritima* L. New Phytol., **41**: 151-64.

Grace, N. H. and Zuckerman, A. (1947). Rapeseed oil. Canadian Chemistry and Process Industries.

Johnson, I. J. and Sass, J. E. (1942). Tetraploidy in *Melilotus alba* induced by colchicine. Proc. Iowa Acad. Sci., **49**: 254.

Julen, G. (1944). Investigations on diploid, triploid and tetraploid luzerne. Hereditas, **30**: 567-82.

Kostoff, D. (1940). Fertility and chromosome length. Correlations between chromosome length and viability of gametes in autopolyploid plants. Jour. Hered., **31**: 33-4.

Levan, A. (1940). Production of tetraploid red clover. Sverig. Utsadesforen Tidskr., **50**: 115-24.

Lewkowitsch, J. and Warburton, J. H. (1922). Chemical technology and analysis of oils, fats and waxes. London: Macmillan & Co., Ltd.

Muntzing, A. (1936). The evolutionary significance of autopolyploidy. Hereditas, **21**: 213-78.

Myers, W. M. (1945). Meiosis in autopolyploid *Lolium perenne* in relation to chromosome behaviour in autopolyploids. Bot. Gaz., **106**: 304-16

Myers, W. M. and Hill, H. D. (1947). Distribution and nature of polyploidy in Festuca elatior. Bull. Torrey Bot. Club, **74**: 99-111.

Peto, F. H. and Boyes, J. W. (1940). Comparison of diploid and triploid sugar beets. Can. Jour. Res. C., **18**: 273-82.

Porter, K. B. and Weiss, M. W. The effects of polyploidy on soybeans. J. A. S. A., **40**: 710-24.

Randolph, L. F. (1935). Cytogenetics of tetraploid maize. Jour. Agr. Res., **50**: 591-605.

——— (1941). An evaluation of induced polyploidy as a method of breeding crop plants. Amer. Nat., **75**: 347-63.

Salomon, E. S. (1944). *Sorghum sudanese* Stapf. tetraploide obtenido por colchicine. Inst. Fitotec. Santa Catalina, 13-16.

SHALYGIN, I. N. (1941). Production of tetraploids in *Lolium* by treating germinating seeds with colchicine. Comp. Rend. Acad. Sci., **30**: 327-9.

STEBBINS, G. L. (1942). Polyploid complexes in relation to ecology and the history of floras. Amer. Natur., **76**: 36-45.

SULLIVAN, J. T. and MYERS, W. M. (1939). Chemical constitution of diploid and tetraploid *Lolium perenne*. J. A. S. A., **31**: 869-71.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIV : SERIES III : JUNE, 1950
SECTION FIVE

A Study of the Mechanism of Reversal of Spinal and Bulbar Reflexes

By R. A. HUGHES, G. W. STAVRAKY, and R. D. TEASDALL

Presented by J. B. COLLIP, F.R.S.C.

IT is known that reflex responses in a limb elicited by means of electric stimulation of afferent nerves may vary with the strength and frequency of the stimulating current, the afferent nerve which is stimulated, posture of the limbs, position of the head and neck, fatigue, administration of various chemicals, and other conditions.

Reflex reversal due to changes in the strength of afferent stimulation was described by Fröhlich (1909) who found that weak ipsilateral stimulation of an afferent nerve caused contraction of the opening muscle of the claw of arthropodes while strong stimulation led to a relaxation of the muscle, the opposite relationship prevailing in the closing muscle of the claw. These results were substantiated by Croisier (1944). Sherrington and Sowton (1911) recording from the limb responses of the isolated quadriceps in the decerebrate cat found that strong electric stimulation of an ipsilateral afferent nerve caused flexion, while weak stimulation of the same nerve resulted in extension of the limb. Hinsey, Ranson and Doles (1930), in an extensive survey, described in the cat numerous instances of reversal of the crossed extensor reflex brought about by changes in the intensity and the frequency of stimulation and by means of stimulation of different afferent nerves. They concluded that in the case of the crossed reflexes, as in the ipsilateral ones, weak stimuli favour extension while stronger ones tend to bring on flexor responses. The mechanism of reversal of reflexes was usually accounted for by the supposed presence of excitatory and inhibitory fibres of different thresholds in the afferent nerves (Sherrington and Sowton (1911a and b); Forbes, Davis and Lambert (1930); Brücke, Hou and Krannich (1931); and others) though as pointed out by Sherrington and Sowton at the time this contention was not the only one possible. Detailed studies of the effect of various frequencies of stimulation upon reflex reversal led Wyss (1940, 1947) to the conclusion that the mechanism of reversal in the case of variations in frequency was a central one. Elaborating this view he suggested the existence of inter-neurones which responded to different rates of stimulation thus leading either to an excitation or to an inhibition of the appropriate motor neurones. In studying the effects of deafferentation upon spinal neurones, it was found by Teasdall and Stavraky (1949) that sensitization by partial denervation resulted in the reversal of proprioceptive reflexes concerned with the regulation of muscular tone (attitudinal postural reflexes or labyrinthine and neck reflexes of the statotonic type). It was shown by Cannon and Haimovici (1939) and by Stavraky (1943) that decentralization causes hyperirritability of the lower motor neurones. In view of the findings of Teasdall and Stavraky it seemed interesting to determine the effect which partial denervation by means of decentralization would have upon the reversal of spinal reflexes. With this in view a systematic study was undertaken of the effects which sensitization of spinal neurones by means of semidecerebration carried out four to six weeks before the experiment would have upon reversal of spinal reflexes. In addition to this, a study of the reversal of some respiratory reflexes was carried out.

METHODS

Twenty-eight cats were used for the investigation of the reversal of spinal reflexes. Decerebration was performed under éther anaesthesia at the intercollicular level while in the spinal preparations the cord was sectioned at the foramen magnum. In twelve of these animals semidecerebration was carried out four to six weeks before the actual experiment, the remainder of the animals being used as controls. Simultaneous recordings from both quadriceps muscles were made. The obturator, hamstrings, and the peroneo-popliteal and posterior tibial nerves were severed bilaterally; the central ends of the latter two nerves were taken on ligatures and used for afferent stimulation, the peroneo-popliteal nerve being the one stimulated as a rule. Drills were inserted into the upper and lower ends of the femora and, with the animal in a supine position, clamped to uprights so that the femora were immobilized almost perpendicularly to the table. The tendons of the quadriceps muscles were divided close to their insertions and the movements were recorded over pulleys on a smoked drum kymograph by means of isotonic levers opposed by 10 grams spring tension. The stimulation of afferent nerves was carried out by means of a square wave stimulator. Variations in intensity of the stimulating current from 0.1 to 100 volts were employed, the frequency range in the present series of experiments being limited to between 10 to 60 per second and the pulse duration set at 0.5 milliseconds.

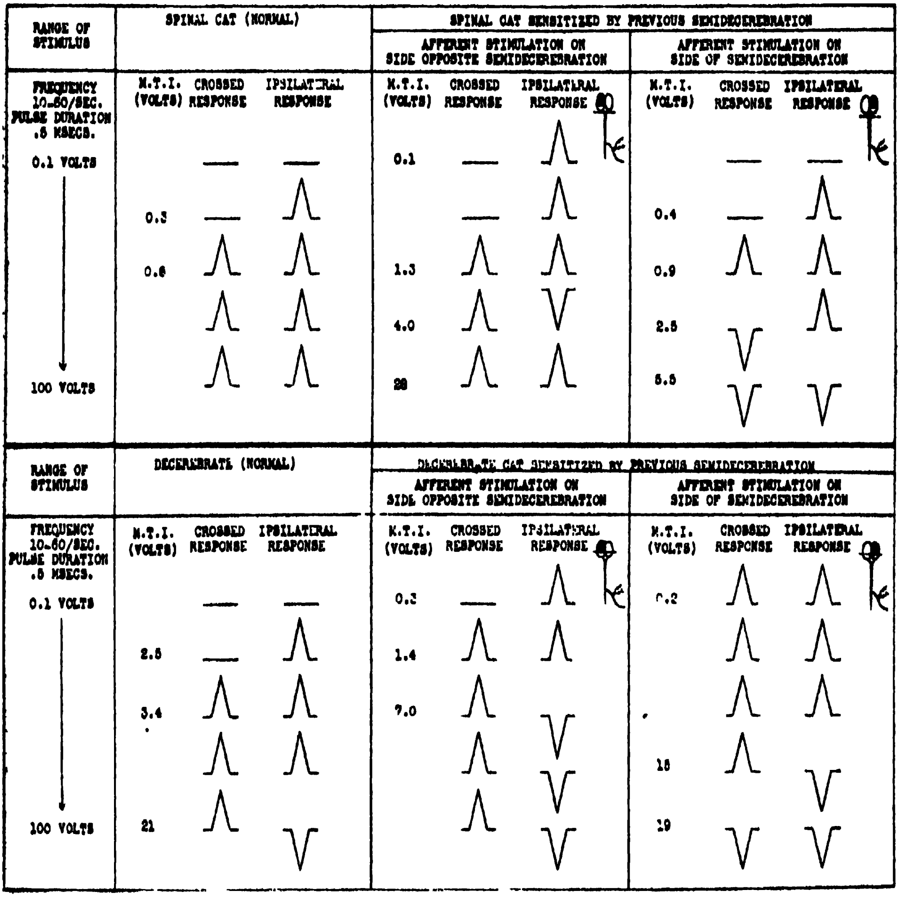
Respiration was studied in seven rabbits in which a diaphragmatic slip was prepared according to Head's technique (Head, 1889). These experiments were performed under urethane anaesthesia (1 cc. of 10 per cent urethane per kg. of body weight injected intravenously). The central ends of the severed vagi nerves were stimulated in the neck with currents of 0.1 to 10 volts intensity, 28 to 60/sec. frequency, and a constant pulse duration of 0.1 millisecond. When both vagi were stimulated simultaneously, two electrodes were attached to the same stimulator and the voltage in such stimulations dropped about 25 per cent from that of the stimulating current applied to each of the nerves separately.

RESULTS

The results obtained in the present series of experiments have been grouped by operative procedure, by the side of stimulation, and by certain ranges of intensity of stimulation applied to the afferent nerve. By referring to a result obtained within a certain range of intensity of afferent stimulation it is meant that a consistent pattern of responses could be produced in an individual animal by one of the voltages lying within the given range, and not that the pattern could be produced throughout the range of the indicated voltages. This was due to the fact that with the technique employed many conditions such as the exact level of transection of the central nervous system, bleeding, operative shock, external temperature, variations in the degree of ventilation, and other conditions resulted in preparations of different responsiveness. Actually, the range of the stimulating current which produced a certain type of response varied considerably in different experiments. However, if each animal is considered individually, a definite pattern of responses was observed as the current intensity (referred to further as CI) was progressively increased.

When intact animals were made spinal or decerebrated immediately before the experiment, the pattern and magnitude of the responses was the same regardless of the side on which the afferent nerve was stimulated. In cats semidecerebrated four to six weeks before the experiment, all the described responses were more pronounced and were elicited with weaker stimulating currents on the decentralized side.

Comparison of reflex responses in the spinal and decerebrate cat: From the diagrammatic representation of the results of the present experiments shown in Figure 1, and from Figure 2, it can be seen that the following pattern of responses to afferent stimulation was elicited in cats made spinal immediately before the experiment: CI ranging between 0.1 and 4 volts in different experiments produced only ipsilateral contraction of the quadriceps. The mean threshold



RANGE OF STIMULUS	SPINAL CAT (NORMAL)			SPINAL CAT SENSITIZED BY PREVIOUS SEMIDECEREBRATION					
				AFFERENT STIMULATION ON SIDE OPPOSITE SEMIDECEREBRATION			AFFERENT STIMULATION ON SIDE OF SEMIDECEREBRATION		
FREQUENCY 10-60/SEC. PULSE DURATION .5 MSECS.	M.T.I. (VOLTS)	CROSSED RESPONSE	IPSILATERAL RESPONSE	M.T.I. (VOLTS)	CROSSED RESPONSE	IPSILATERAL RESPONSE	M.T.I. (VOLTS)	CROSSED RESPONSE	IPSILATERAL RESPONSE
0.1 VOLTS		—	—	0.1	—	Λ		—	—
	0.3	—	Λ		—	Λ	0.4	—	Λ
	0.8	Λ	Λ	1.3	Λ	Λ	0.9	Λ	Λ
		Λ	Λ	4.0	Λ	V	2.5	V	Λ
100 VOLTS		Λ	Λ	28	Λ	Λ	5.5	V	V

RANGE OF STIMULUS	DECEREBRATE (NORMAL)			DECEREBRATE CAT SENSITIZED BY PREVIOUS SEMIDECEREBRATION					
				AFFERENT STIMULATION ON SIDE OPPOSITE SEMIDECEREBRATION			AFFERENT STIMULATION ON SIDE OF SEMIDECEREBRATION		
FREQUENCY 10-60/SEC. PULSE DURATION .5 MSECS.	M.T.I. (VOLTS)	CROSSED RESPONSE	IPSILATERAL RESPONSE	M.T.I. (VOLTS)	CROSSED RESPONSE	IPSILATERAL RESPONSE	M.T.I. (VOLTS)	CROSSED RESPONSE	IPSILATERAL RESPONSE
0.1 VOLTS		—	—	0.3	—	Λ	0.2	Λ	Λ
	2.5	—	Λ	1.4	Λ	Λ		Λ	Λ
	3.4	Λ	Λ	7.0	Λ	V		Λ	Λ
		Λ	Λ		Λ	V	15	Λ	V
100 VOLTS	21	Λ	V		Λ	V	19	V	V

FIGURE 1.—Diagrammatic representation of reflex responses as observed in both quadriceps on electric stimulation of the peroneo-popliteal or posterior tibial nerve with increasing intensities in ordinary spinal and decerebrate cats and in the same preparations after sensitization by semidecerebration four to six weeks previous to the experiment:

MTI—"Mean threshold intensity" of the stimulating current.

"Crossed Response"—indicates response of the quadriceps contralateral to the stimulated nerve.

"Ipsilateral Response"– indicates response of the quadriceps on the side of the stimulated nerve.

Horizontal line—represents the absence of any response.

Symbol in the form of an inverted "V"—represents contraction of the quadriceps.

"V" shaped symbol—represents relaxation of the quadriceps.

(On the small diagrammatic illustrations of the central nervous system the horizontal line represents the level of transection at the beginning of the experiment, while the area blotted out in black shows the side of the preceding semidecerebration. The peroneo-popliteal nerve and the electrodes are shown in relation to the semidecerebration and to the responses obtained.)

intensity (referred to further as MTI) was 0.3 volts for the whole group in the case of this ipsilateral response. On the other hand, greater CI, beginning from 0.5 and going up to 100 volts, MTI—0.8 volts, caused bilateral contraction of the quadriceps. Six experiments of this type were performed, the pattern of the responses being quite uniform in all regardless of whether the peroneo-popliteal or posterior tibial nerves were being stimulated. Thus, in cats made spinal immediately preceding the experiment, reversal of the extensor reflexes was not encountered in this series.

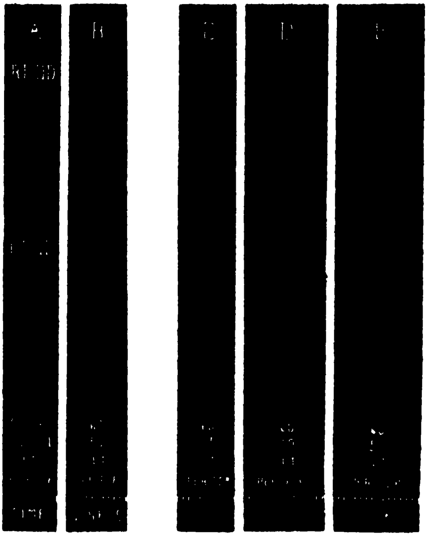


FIGURE 2.—Comparison of responses to increasing intensities of stimulation of the left peroneo-popliteal nerve in an ordinary spinal and decerebrate cat. (LT. QD.—left quadriceps; RT. QD.—right quadriceps; FR.—frequency of stimulating currents per second; VO.—voltages of stimulating current; LT. PER.-POP.—left peroneo-popliteal nerve; RT. PER.-POP.—right peroneo-popliteal nerve. Abbreviations on all following tracings are the same as in Figure 2.)

A and B—spinal cat.

C, D, and E—decerebrate cat.

In ten experiments in which decerebration at the intercollicular level was carried out, a different sequence of effects took place when the intensity of the stimulating current was progressively increased. CI ranging between 0.1 and 7 volts, with MTI of 2.5 volts, produced ipsilateral contractions in eight experiments, while in two of the animals bilateral responses were seen to occur from the start even with the weakest currents. With CI ranging between 1 and 20 volts and MTI of 3.4 volts, bilateral contractions of the quadriceps muscles were seen to replace the unilateral responses in the eight aforementioned experiments in which the responses to weaker stimulation were localized to the quadriceps on the same side as the stimulation. A further increase in CI—5 to 100 volts (MTI—21 volts) resulted in a crossed contraction of the quadriceps associated with an ipsilateral relaxation of this muscle. In two animals of this series spontaneous bilateral clonus developed some time during the experiment; in these cats stimulation of the afferent nerve resulted in a bilateral relaxation of the quadriceps muscle. This effect was not constantly reproducible, however, and occurred only when the stimulation was superimposed upon the clonus. In two of the ten experiments on decerebrate cats, after obtaining a repeatable maximal response which consisted of a contraction of the quadriceps contralateral to the stimulated nerve and of a relaxation on the side of stimulation, the animals were transected below the medulla. With this procedure the response changed to a bilateral contraction of the quadriceps muscles and stayed that way until the end of the experiment despite the increase of the CI up to 100 volts.

Comparison of reflex responses in ordinary spinal cats with those of spinal cats sensitized by semidecerebration of four to six weeks' standing: As described in the foregoing section, in cats with a high spinal transection carried out shortly before the experiment, two variations of responses to afferent stimulation were seen: (1) ipsilateral contraction of the quadriceps when the intensity of the stimulating current was low, and (2) bilateral contraction when the intensity of the current was increased. In six cats the same experiment was repeated following a semidecerebration.

(a) *Effect of stimulation of an afferent nerve on the sensitized side of the spinal cord*: As seen in Figure 1 and in Figure 3, weak stimulation of an afferent nerve on the side opposite to the semidecerebration produced an ipsilateral contraction of the quadriceps. In all six cats this took place with a CI ranging between 0.1 and 10 volts, the MTI being 0.1 volt. With an increase of the intensity of stimulation (CI—0.5 to 20 volts, MTI—1.3 volts) bilateral contraction of the quadriceps muscles took place in five animals. In the remaining one a contraction of the quadriceps contralateral to the stimulated nerve occurred, coupled with a relaxation of the quadriceps on the side of the stimulation. This took place directly after the ipsilateral response without the intermediate effect of a bilateral contraction. When the CI was increased to from 1.5 to 25 volts (the MTI being 4 volts) this divided response of ipsilateral relaxation and contraction contralaterally developed in three of the six tested animals. With still greater voltages (CI—25 to 100 volts, MTI—28 volts) the response reverted once again to a bilateral contraction.

(b) *Effect of stimulation of an afferent nerve contralateral to the sensitized half of the spinal cord*: Stimulation of an afferent nerve on the side of the semidecerebration produced a somewhat different pattern of responses. In four of the six animals an ipsilateral contraction of the quadriceps could be elicited with CI ranging between 0.1 and 5 volts, the MTI being 0.4 volts, while in the remaining two animals a bilateral contraction appeared from the start. With CI ranging from 0.2 to 100 volts, and MTI being 0.9 volts, bilateral contractions of the quadriceps muscles occurred in all six animals. On progressively increasing the intensity of the stimulating current (CI—0.5 to 100 volts, MTI—2.5 volts) a relaxation of the opposite quadriceps occurred in five of the six cats, this being coupled with a

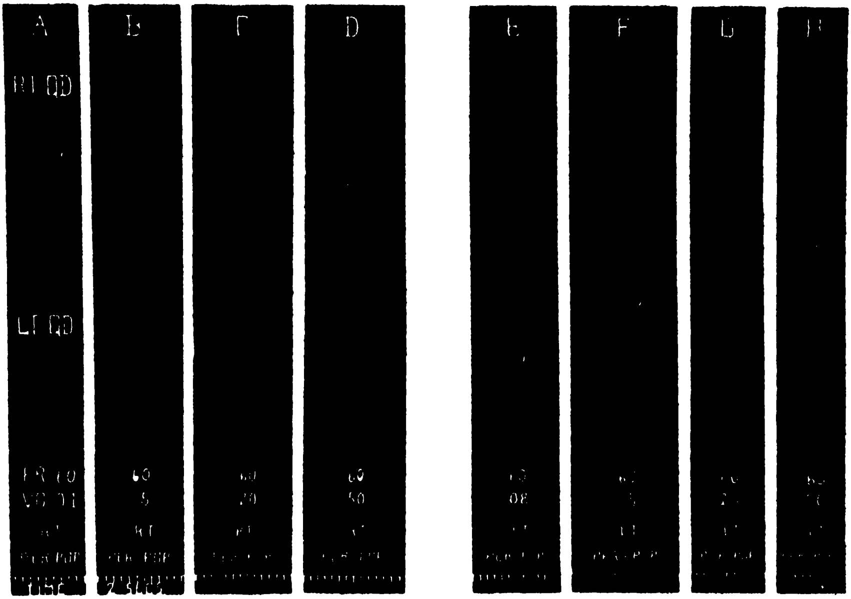


FIGURE 3.—Comparison of the responses to increasing intensities of afferent stimulation on two sides of a spinal cat sensitized by a left semidecerebration of 35 days' duration.

Tracings A, B, C, and D—show responses of the quadriceps muscles to the stimulation of the *right* peroneo-popliteal nerve.

Tracings E, F, G, and H —show responses of the quadriceps muscles to stimulation of the *left* peroneo-popliteal nerve.

contraction of the muscles on the side of the stimulation. A further increase in CI—1 to 100 volts (MTI—5.5 volts) resulted, in two experiments, in a bilateral relaxation of the quadriceps muscles. However, even in this case, the effect on the side of the stimulation was often not a pure relaxation but an initial contraction with a relaxation at the height of the effect of stimulation, this again followed by a "rebound" type of contraction when the stimulation was discontinued. Thus, sensitization of one half of the spinal cord by preceding semidecerebration predisposed the sensitized side of the spinal cord to the appearance of reversal of the extensor reflexes on that side. This effect was more constantly demonstrable when the afferent nerve was stimulated on the control side, in some experiments leading even to a bilateral inhibition of the extensors.

Comparison of the reflex responses in ordinary decerebrate cats with those of decerebrate cats sensitized by semidecerebration of four to six weeks' standing. As stated earlier, in the ordinary decerebrate cats, three types of responses were encountered when the stimulating current was progressively increased: (1) ipsilateral, then (2) bilateral contraction of the quadriceps, followed by (3) a crossed extensor response in association with an ipsilateral relaxation of the quadriceps. The same experiment was repeated in six cats in which semidecerebration was carried out with the following results:

(a) *Effect of stimulation of an afferent nerve on the sensitized side of the spinal cord in a decerebrate cat*: As shown in Figure 1 and Figure 4 weak stimulation of an afferent nerve (CI—0.1 to 0.5 volts, MTI—0.3 volts) resulted again in an ipsilateral contraction of the quadriceps. An increase in the intensity of afferent stimulation (CI—0.5 to 100 volts, MTI—1.4 volts) resulted in the appearance of a bilateral contraction of the quadriceps muscles. Both these types of responses were seen to occur in all the animals tested. With further increases in CI the crossed extensor response persisted but became associated with a relaxation of the ipsilateral quadriceps, this taking place in five of the animals when the CI ranged between 5 and 100 volts and the MTI was 7 volts. In one cat a bilateral contraction persisted right up to current intensities of 100 volts. When the responses in this group of experiments were compared with those of the ordinary decerebrate animals, no difference in the type of response could be seen, but all the three responses were obtained with a greater regularity and ease, and often within a lower range of stimulating currents.

(b) *Effect of stimulation of an afferent nerve contralateral to the sensitized half of the spinal cord in a decerebrate cat*: Stimulation of the afferent nerve on the side of the semidecerebration resulted, in the decerebrate cats, as in the spinal preparations, in a different pattern of responses from the one seen when the afferent stimulation was carried out on the opposite side. No unilateral response could be produced in this type of experiment, even the weakest stimuli (CI—0.1 to 10 volts, MTI—0.2 volts) causing a bilateral contraction of the quadriceps muscles in all of the tested animals. A crossed extensor response coupled with a relaxation of the quadriceps on the side of the stimulation occurred again in every one of the cats when the CI was increased, (CI—1 to 100 volts, MTI—15 volts). An increase in the intensity of the afferent stimulation beyond the range in which this effect was obtained resulted in a relaxation of the quadriceps muscles on both sides in four of the six experiments, this taking place with CI—10 to 100 volts and MTI—19 volts.

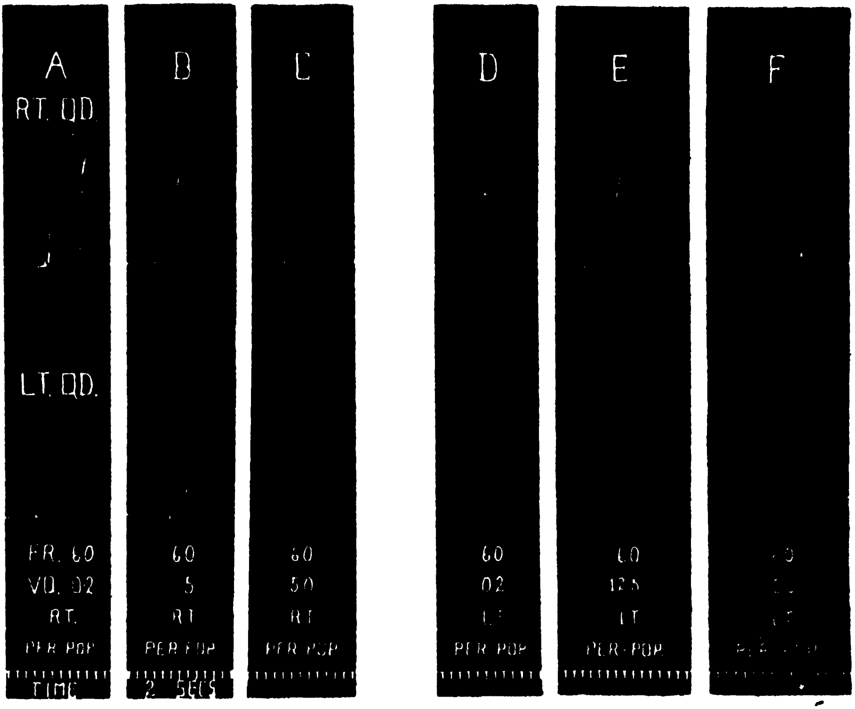


FIGURE 4.—Comparison of the responses to increasing intensities of afferent stimulation on two sides of a decerebrate cat sensitized by a left semidecerebration of 41 days' duration.

Tracings A, B, and C—show responses of the quadriceps muscles to stimulation of the *right* peroneo-popliteal nerve.

Tracings D, E, and F—show responses of the quadriceps muscles to stimulation of the *left* peroneo-popliteal nerve.

Throughout this study, besides the reversals seen with increases in the intensity of the stimulating current, numerous instances of reversals were observed due to changes in the frequency of stimulation. However, no special study of these reversals was made at the present time and therefore a description of the influence of changes in frequency is not presented here.

ANALYSIS OF THE RESULTS

As stated earlier, two views are prevalent in the literature regarding the mechanism of reflex reversal, the most commonly accepted one assumes the existence of two sets of fibres of different thresholds in the peripheral afferent nerves leading to different effects depending on which type of fibre is stimulated. The other view maintained by Wyss (1940, 1947) in relation to trequency reversals postulates the existence of two types of intercalated neurones in the central nervous system, excitatory and inhibitory ones leading to different responses depending upon which type of interneurone is excited. Though mixed nerves were stimulated in the present study and the admixture of effects of stimulation of different fibres cannot be completely excluded, an analysis of the various experiments leads to the conclusion that in the case of variations in intensity of the stimulating current as well as in the case of frequency reversals, predominantly a central mechanism must be involved. This conclusion is based on the following facts:

1. The conversion of an ordinary decerebrate preparation into a spinal one by means of a section of the cord below the medulla led to the disappearance of an ipsilateral relaxation of the quadriceps and to the establishment of a bilateral contraction in spite of the fact that the intensity of the stimulating current remained unchanged.

2. Sensitization of the spinal neurones by preceding decentralization (semidecerebration) resulted in a facilitation of inhibitory processes mostly on the corresponding side of the cord leading to reflex reversals under experimental conditions under which, normally, reflex reversals did not occur. This can be seen throughout the comparison of responses in the ordinary spinal and decerebrate preparations with those in animals previously sensitized by semidecerebration (Figures 1, 2, 3, and 4).

3. A particularly convincing example of central reflex reversal is presented in Figure 5. In this experiment performed on a decerebrate cat sensitized by a preceding left semidecerebration a series of stimulations of the left peroneo-popliteal nerve with a current of 0.4 volt intensity resulted in a series of bilateral contractions of the quadriceps

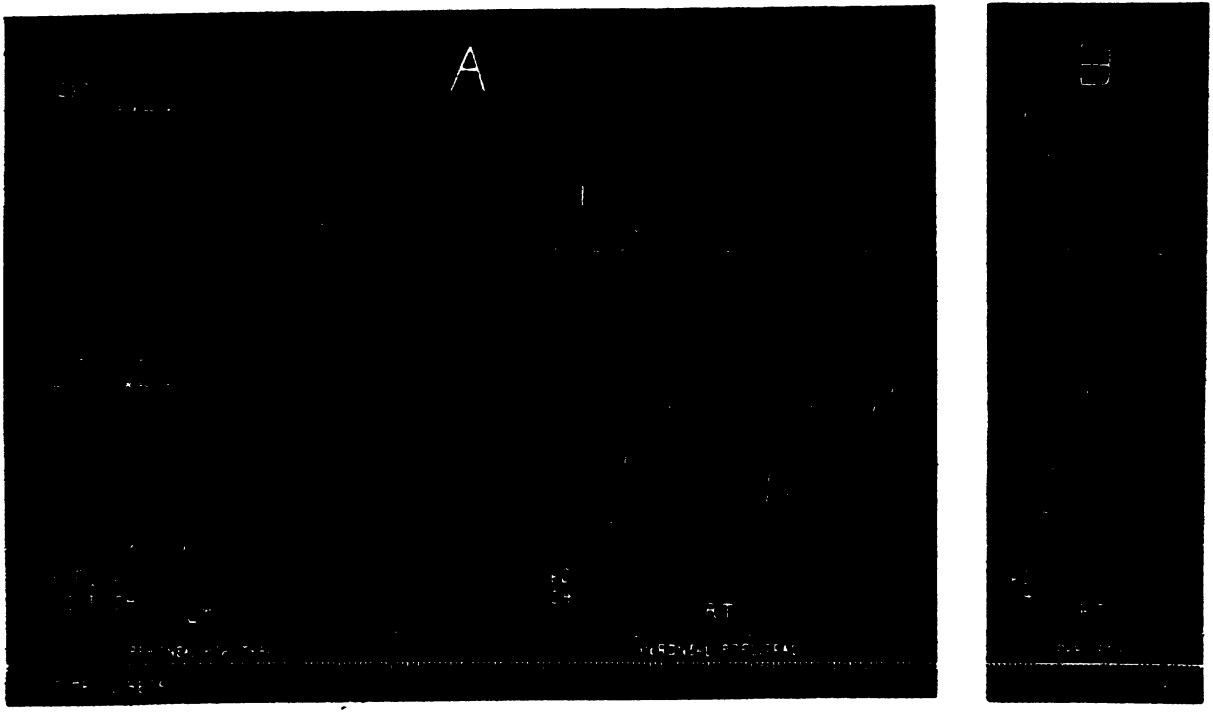


Figure 5.—Progressive summation of central excitation resulting in a unilateral reversal of the reflex which takes place on the sensitized side of the spinal cord. Left semidecerebration carried out 35 days previously.

A—Effect is produced in decerebrate cat by stimulation of the *right* peroneo-popliteal nerve with a weak current. (Stimulation of the *left* nerve with the same current causes only bilateral contractions.)

B—Repetition of the stimulation of the *right* peroneo-popliteal nerve 28 seconds after the preceding stimulation.

muscles, the contractions being more pronounced on the right side, this corresponding to a crossed extensor response. A similar series of stimulations applied to the right peroneo-popliteal nerve resulted in several progressively increasing contractions of the quadriceps on the side of stimulation. As the stimulations were continued the "central excitatory state" reached its maximum and a reversal of the reflex occurred, this manifesting itself in a series of relaxations of the muscle. The total response gave the impression that the stimulating current was being progressively increased though it was kept constant throughout the series of stimulations. An example of an analogous gradual

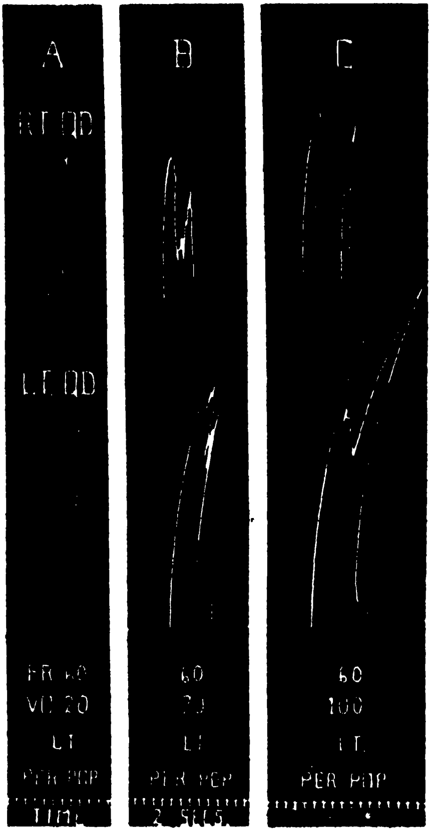


FIGURE 6.—Development of inhibition at the height of afferent stimulation in a spinal cat sensitized by a preceding left semidecerebration of 40 days' duration. Effect becomes more pronounced with increasing intensities of stimulating current and in tracing *C* is bilateral with predominance on the sensitized side. Note that the "rebound" developed parallel with the inhibition.

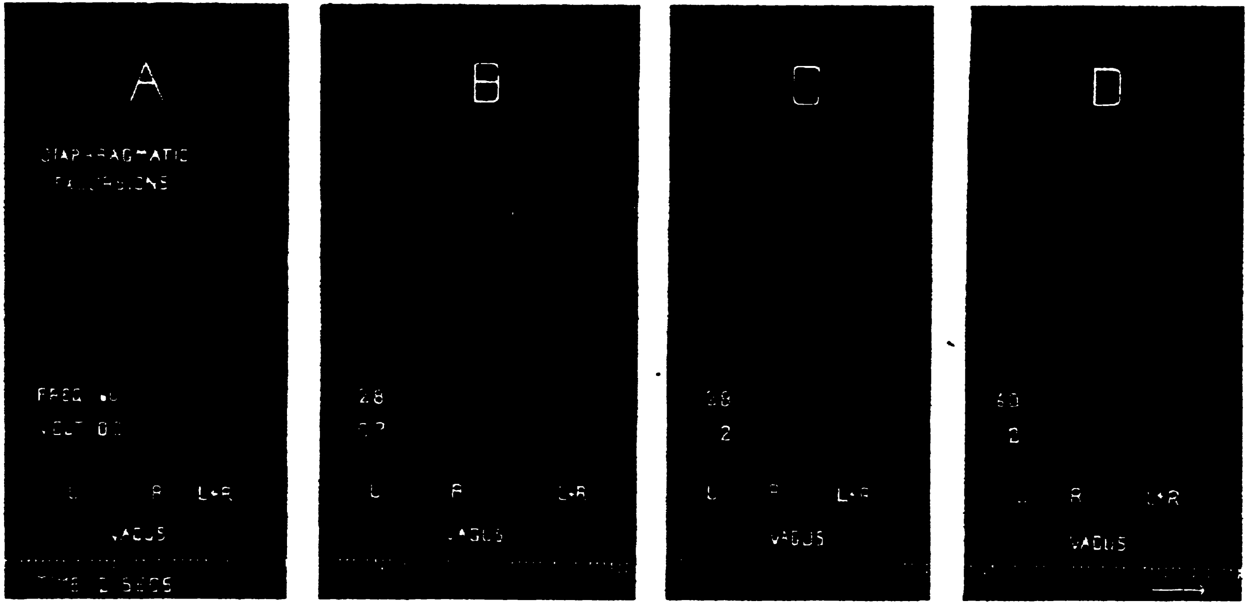


FIGURE 7.—Central summation of the effects of stimulation of both vagi nerves in the neck in the rabbit recorded by means of a diaphragmatic slip.

A—Stimulation of the vagi nerves separately is ineffective but effective summation takes place when both nerves are stimulated with the same current simultaneously (L and R).

B—Summation of the effects of minimal stimulation results in an acceleration of the respiratory movements and in greatly reduced excursions, almost an arrest in inspiration (L and R).

C and *D*—Somewhat stronger stimulation of each nerve separately results in an inspiratory type of response (in *D* gradually sinking to an in-between position as stimulation progresses) with a reduction in amplitude and acceleration of respiratory movement. Simultaneous stimulation of both vagi results in an expiratory arrest (L and R).

summation of excitation which resulted in the appearance of a reversal when the CI was increased is shown in Figure 6. In this experiment, progressive increases in the intensity of afferent stimulation resulted in a more marked development of relaxation of the quadriceps in the middle of each period of stimulation, while at the beginning and after the discontinuance of the stimulation the muscles responded by contractions. Instances of complete reversal with increases of the intensity of the stimulating current may be seen in Figures 2, 3, and 4.

4. It may be added that in many instances in spinal and decerebrate preparations, both sensitized by previous semidecerebration and not, with deterioration of the general condition of the animal which occurred during a prolonged experiment, the same current intensities which in the beginning of the experiment caused various inhibitory responses, later in the experiment produced only contractions of the quadriceps muscles; again not the threshold of the afferent fibres but the state of the central nervous system determining the type of response.

5. Finally, in order to demonstrate that reversal of a reflex may be brought about by central summation of excitation without increasing the strength or altering the frequency of the stimulating current and without any preceding sensitization by denervation of the spinal neurones, the experiment shown in Figure 7 was carried out. In this experiment, performed successfully in six out of seven rabbits, when currents ranging in intensity from 1 to 10 volts were applied to the central end of each vagus nerve separately, they produced an inspiratory and sometimes an in-between type of response with a reduction in amplitude and acceleration of the respiratory movements. Applied to both vagi simultaneously, the same currents caused an arrest of breathing of an expiratory nature (Tracings C and D of Figure 7). Wyss (1940) also obtained a reflex reversal of respiration without changing the intensity or the frequency of the stimulating current by applying in phase and out of phase stimulation to both vagi nerves.

Along the same line of reasoning it may be mentioned that strychnine, chloroform, and other agents which have a central action, markedly affect the reversal of reflexes (Owen and Sherrington (1911), Sherrington and Sowton (1911c), Brown (1911, 1914), Forbes (1921, 1922)) as well as does the onset of fatigue (Sherrington and Sowton (1911a and b), and Verzar (1923)).

DISCUSSION

The results of the present study warrant three generalizations:

1. Reversal of reflexes in extensor muscles occurs with increases in the intensity of afferent stimulation, that is, when the CI reaches a

critical point relaxation of the muscle replaces the contraction on the side of stimulation and under certain conditions contralaterally as well.

2. Reversals occur more readily in decerebrate preparations than in spinal animals and conversion of a decerebrate preparation into a spinal one during the same experiment leads to the disappearance of reflex reversals provided other conditions such as the CI remain unaltered.

3. Sensitization by preceding partial denervation of the spinal centre predisposes the corresponding neurones to the occurrence of reflex reversals.

The first conclusion is in general agreement with the observations of Sherrington and Sowton (1911a and b) and with those of Hinsey, Ranson, and Doles (1930). The second one, in its application to reciprocal innervation, is generally accepted. In connection with the third conclusion, particularly interesting is the fact that Hinsey, Ranson, and Doles saw reversal of the crossed extensor reflex in cats made spinal up to fifteen days before the experiment, thus carrying out observations on reflex responses in a sensitized spinal cord. In the present series of acutely transected animals reversal of the crossed extensor response did not occur, but it did take place in animals which were semidecerebrated four to six weeks before the experiment and then it occurred on the sensitized side of the cord. Preceding semidecerebration, however, brought out reflex reversals not only on the appropriate side but also to a lesser extent contralaterally, therefore semidecerebration facilitated in general the reflex responses. A similar impression was gained by Cannon, Rosenbleuth, and Ramos (1945). These authors came to the conclusion that both excitatory and inhibitory processes are increased by a semisection of the spinal cord of some standing.

The ability of spinal neurones, after sensitization by unilateral deafferentation, to maintain a prolonged state of excitation leading to a fusion of responses brought on by a succession of stimulations was noted previously by Drake and Stavraky (1948). The latter investigators showed a fusion of several successive knee jerks induced by stimulation of the opposite quadriceps, and a more prolonged response to asphyxia on the deafferented side of the cord in the spinal cat. These observations are in keeping with the fusion of effects of individual stimulations shown in Figures 5 and 6.

Turning to the mechanism of reflex reversal, the considerations presented in the analysis make it improbable that the phenomenon depends upon the stimulation of peripheral nerve fibres of different thresholds with different current intensities. The suggestion of Wyss

(1940, 1947) that the reflex reversals caused by different frequencies of the stimulating current depend upon the presence of intercalated neurones of various thresholds in the central nervous system should therefore be considered in its application to the different intensities of stimulation. Acceptance of this explanation, however, would necessitate the assumption of the existence of several sets of intercalated neurones, one set responding to certain frequencies, another to certain intensities of stimulation. Besides this, such an explanation would not readily account for reflex reversals caused by sensitization by means of partial denervation; also, the reflex reversals caused by various chemical agents and by fatigue would be difficult to interpret on the basis of this hypothesis.

Another hypothesis of reflex reversal as it applies to the instance of "reciprocal innervation" was suggested by Gasser (1937) who visualized the arrival of excitatory and inhibitory impulses via different fibres to several intercalated neurones. According to Gasser's view the numerical preponderance of discharging synaptic terminations on these intercalated neurones determines whether the ultimate effect would be excitation or inhibition of the motoneurones.

In a search for an explanation of the mechanism involved in reflex reversal which would account for all the experimental findings presented in this investigation, the various instances of dosage reversal of the action of acetylcholine on peripheral structures came to mind. Evidence has accumulated in recent years which indicates that acetylcholine when introduced in small quantities causes vasodilatation, salivary and gastric secretion, acceleration of the heart, inhibition of intestinal motility, and contraction of the striated muscles. However, when administered in excessive amounts, particularly by the intra-arterial route or by local application acetylcholine causes the opposite effects (Spadolini (1948), McDowall (1946), Bulbring and Burn (1949), Burn and Vane (1949), Welsh (1948), Morton and Stavraky (1949), Stavraky and Graham (1950), and others). Also numerous instances of reversal of the effect of stimulation of peripheral autonomic nerves are known to occur, the reversal being due to variations in the intensity of stimulation and often taking place under special experimental conditions (Fröhlich and Löewi (1906), Stavraky (1934), Spadolini (1948), and others).

All these instances of reversal of the effect of chemical and nervous stimulation in peripheral structures depend upon the quantity of the stimulating agent or upon the strength of stimulation of the efferent nerve and upon the state of the peripheral structure, and what is important, they take place in the absence of intercalated neurones which complicate the studies upon the central nervous system. Except for the latter however, the conditions under which peripheral reversals take place are not unlike those which are encountered in the central nervous system. Here too the reflex reversals are found to depend upon the intensity of the stimulation of the afferent nerve and upon the state of sensitivity of the centre.

Thus, as far as extensor reflexes in the decerebrate cat are concerned, weak stimulation of the peronco-popliteal nerve leads to an ipsilateral contraction of the quadriceps, while stronger stimulation leads to a bilateral response in these muscles, and very strong stimulation to a relaxation of the ipsilateral quadriceps coupled with a contraction of the opposite one. Sensitization by previous partial denervation of one side of the spinal cord leads to the relaxation of the quadriceps also on the side opposite to the stimulated nerve when the stimulation is carried out contralaterally to the sensitized region—a bilateral relaxation occurring on stimulation of the peroneo-popliteal nerve. Therefore, it seems that the number of nerve impulses which reach a given group of neurones within a given time determines the type of response of these neurones provided other conditions remain unaltered. Furthermore, a certain optimal number of impulses lead to excitation while an excessive number of impulses lead to an inhibition.

However, the amount of excitation in the central nervous system must be graded so that the ipsilateral group of neurones can respond alone to the weakest stimulation leading to an ipsilateral contraction of the quadriceps; then when the strength of the stimulation is increased this ipsilateral group of neurones responds by a reversal from excitation to inhibition—this leading to a relaxation of the ipsilateral quadriceps, while the opposite muscle responds by a contraction. It seems that this grading of the amount of excitation can be most satisfactorily accounted for by the assumption that the ipsilateral neurones receive numerically more nerve fibre terminations than the ones on the opposite side of the spinal cord.

This concept makes it possible to account for the finding that ipsilateral inhibition of the quadriceps is changed into a contraction by the conversion of an animal from a decerebrate preparation into a spinal one by a second transection of the central nervous system. In decerebrate animals, stimulation of the peroneo-popliteal nerve results in a given number of impulses arriving at the spinal neurones which receive at the same time impulses from the vestibular centre due to labyrinthine stimulation and other impulses which reach the spinal neurones via the extrapyramidal system. Convergence of all these impulses on the spinal neurones causes an over-excitation of the latter which results in their inhibition. When the spinal cord is sectioned below the medulla the facilitating effect of the descending impulses is abolished and stimulation of the peroneo-popliteal nerve alone is now insufficient to produce inhibition. Accordingly, the response of the ipsilateral quadriceps in a spinal animal is converted into a contraction.

A number of reflex reversals mentioned in the literature which are caused by the position of the head and neck (Socin and Van Leeuwen (1914), Beritoff (1915), Girndt (1926), Pollock and Davis (1924), and Pi-Suner and Fulton (1929), and others), and by the posture of the limbs (Gergens (1876), Sherrington (1900, 1905a and b), Von Uexkull (1904), Magnus (1909, 1910), Brown (1911)) may be accounted for in this manner.

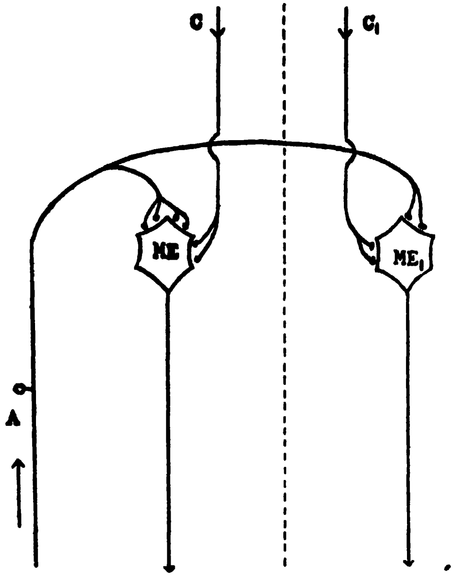


FIGURE 8. Diagrammatic representation of the proposed explanation of the localization of reflex reversal based on the variation in the number of impulses reaching effector neurones.

A—Afferent fibre; ME—Extensor motoneurone ipsilateral to the stimulated afferent fibre; ME1—Extensor motoneurone on the opposite side of the spinal cord; C and C1—Descending fibres from higher levels of the central nervous system (scheme omits interneurones and does not try to express actual numerical relations of end-bulbs to motoneurones).

The greater ease of reflex reversal which takes place after sensitization by partial denervation falls into the group of responses in which a greater effectiveness of the stimulation leads to a reversal.

The latter is achieved however, not by an increased number of afferent impulses converging upon the neurones but by a change in the sensitivity of the neurones themselves to the arriving impulses. This causes the neurones to become more easily overstimulated and consequently inhibited.

Deterioration in the general condition of the animal may lead to the opposite condition—the disappearance of an inhibitory effect of the afferent stimulation, this again being a change in the state of the neurones which manifests itself in a lowered irritability. Consequently, the same number of nerve impulses which early in the experiment led to an inhibition now is not sufficient to produce this effect—a contraction of the quadriceps replacing relaxation. However, a weakening of the animal may conceivably result also in a lessened number of nerve impulses being originated by the same stimulus in the peripheral nerve.

Arising as a logical extension of the accumulating knowledge about the reversal of effects caused by chemical agents in peripheral structures, the suggested concept of the mechanism of reflex reversal from excitation to inhibition may be most easily explained by the increased concentration of a chemical transmitter. However, this does not necessarily presuppose chemical transmission of excitation at the synapses as the only possible theory of transmission to which the concept may be applied. It is felt that the suggested mechanism of reflex reversal may be applied to the electrical theory of synaptic transmission as well as to the chemical theory. If this view is favoured it may be supposed that an appropriate number of nerve impulses causes excitation of the neurones, while the arrival of an excessive number of nerve impulses at the synaptic junctions over a great multitude of terminations results in a widespread depolarization of the overexcited neurones and renders them refractory, this expressing itself in the development of inhibition. Such a process would be conceivably similar in its nature to that which takes place in the case of inhibition of anterior horn cells by antidromic stimulation which was demonstrated by Eccles (Creed, *et al.* (1938), and Eccles (1931)).

Based either on a chemical or on an electrical theory of transmission the concept maintains that inhibition is the ultimate stage of overexcitation. This view makes it possible to account for many known instances of inhibition which occur in the central nervous system and to reconcile the co-operative action of the flexors and extensors which is seen often in intact limbs with the principle of reciprocal innervation demonstrated by Sherrington (Creed, *et al.*, 1938) in isolated groups of muscles, the two responses depending on different degrees of stimulation. Similarly, "rebound" may be thought of as being a contraction caused by a subsiding excitatory process which during the actual stimulation was strong enough to cause a reversal in all or some of the neurones, these neurones during the resolution of the hyperexcitation going through a stage when active discharges from them take place.

On the clinical side the concept may account for the conversion of the physiological extension (downward movement of the large toe) which occurs when the sole of the foot is stroked in normal human beings into a physiological flexion, or as it is also called "dorsiflexion" of the large toe—movement upward (Babinski sign, Fulton and Keller (1932)) when the sole of the foot is stroked in a patient with an upper motor neurone lesion. In this case the neurological lesion leads to a sensitization of the reflex arc by decentralization, causing a reversal of the reflex. Another possible example of a reversal, this one of a strictly physiological type, may be given: the general wilting which takes place in extreme fright and is very marked in some species of animals, such as the rabbit and some birds, and is known to occur also in man, may be interpreted as being the ultimate result of over-excitation and a general reversal of all the reflex activity.

SUMMARY AND CONCLUSION

1. Reflex reversal in both quadriceps muscles brought about by varying the intensity of afferent stimulation was studied in sixteen previously intact spinal and decerebrate cats, and in twelve animals sensitized by an aseptic semidecerebration of four to six weeks' standing.

2. In the previously intact animals increases in intensity of stimulation of the peroneo-popliteal or of the posterior tibial nerve led to a definite pattern of responses: ipsilateral, then bilateral contraction of the quadriceps muscles occurred in spinal preparations, while a sequence of ipsilateral, bilateral, then crossed contraction and ipsilateral relaxation took place in decerebrate animals with an increasing intensity of afferent stimulation.

3. Sensitization of the corresponding spinal centres by preceding semidecerebration greatly predisposed these centres to the occurrence of reflex reversals, this resulting in unilateral or bilateral relaxation of the quadriceps muscles on stimulation of the peroneo-popliteal nerve, the difference in effect depending upon the side on which the stimulation was carried out. Stimulation on the sensitized side resulted in ipsilateral inhibition, while stimulation on the opposite side led to a relaxation of both the quadriceps muscles, this taking place both in spinal and in decerebrate preparations.

4. An analysis of these results was carried out and experimental evidence presented to show that the observed reflex reversals depended upon a central mechanism and not solely upon fibres of different thresholds in the peripheral afferent nerve which responded to different intensities of stimulating currents. This analysis was extended to the effect of vagal stimulation on the respiratory centre carried out in seven rabbits with similar results.

5. The reversal from excitation to inhibition which occurs with increasing intensities of stimulation is regarded as being due to over-excitation of the neurones. The phenomenon is compared with the action of acetylcholine on peripheral structures on which it is known that excessive quantities of acetylcholine cause reverse effects from those induced by acetylcholine when it is introduced in physiological amounts.

6. The localized nature of reflex reversals in the central nervous system, that is, the occurrence of a reversal of one reflex (reversal of the response of the quadriceps on one side of the spinal cord) is accounted for by the numerical difference in the distribution of the afferent terminations, while the greater susceptibility to reversals of the spinal neurones sensitized by previous partial denervation is supposed to depend upon the greater ease with which supersensitive neurones may become overstimulated as compared to the normal ones.

It is felt that the presented concept of the mechanism of reflex reversal may prove useful in the interpretation of many clinical neurological signs and possibly will contribute to the understanding of the nature of central inhibition.

ACKNOWLEDGMENT

The work was carried out in the Department of Physiology, University of Western Ontario, and was aided by grants from the National Research Council of Canada and by a national Mental Health grant.

REFERENCES

Beritoff, J. S. (1915). Quart. Jour. Exper. Physiol., **9**: 199.

Brown, T. Graham (1911). Quart. Jour. Exper. Physiol., **4**: 273.

——— (1914). Jour. Physiol., **48**: 18.

Brücke, E. T., Hou, C. L. and Krannich, E. (1931). Pflüger's Arch. **227**: 733.

Bulbring, E. and Burn, J. H. (1949). Jour. Physiol., **108**: 6P.

Burn, J. H. and Vane, J. R. (1949). Jour. Physiol. **108**: 104.

Cannon, W. B., and Haimovici, H. (1939). Amer. Jour. Physiol., **126**: 731.

Cannon, W. B., and Rosenblueth, A. (1949). The supersensitivity of denervated structures. New York: Macmillan Co., p. 153.

Cannon, W. B., Rosenblueth, A. and Ramos, J. Garcia (1945). Arch. Inst. Cardiol. Méx., **15**: 327.

Creed, R. S., Denny-Brown, D., Eccles, J. C., Liddell, E. G. and Sherrington, C. S. (1938). Reflex activity of the spinal cord. London: Oxford Univ. Press, H. Milford. (Antidromatic stimulation, pp. 38-40; Reciprocal innervation, p. 84.)
Croisier, M. (1944). Helv. Physiol. Acta, **2**: 97.
Drake, C. G. and Stavraky, G. W. (1948). Jour. Neurophysiol., **11**: 229.
Eccles, J. C. (1931). Proc. Roy. Soc., **107B**: 557.
Forbes, A. (1921). Amer. Jour. Physiol., **55-56**: 273.
——— (1922). Physiol. Rev., **2**: 361.
Forbes, A., Davis, H. and Lambert, E. (1930). Amer. Jour. Physiol., **95**: 142.
Fröhlich, A. and Löewi, O. (1906). Zentralbl. f. Physiol., **20**: 229.
Fröhlich, F. W. (1909). Ztschr. f. allg. Physiol., **9**: 55.
Fulton, J. F. and Keller, A. D. (1932). The sign of Babinski. Springfield, Ill. and Baltimore, Md.: C. C. Thomas, pp. 3-14.
Gasser, H. S., (1937). Harvey Lect., p. 169.
Gergens, E. (1876). Pflüger's Arch., **14**: 340.
Girndt, O. (1926). Pflüger's Arch., **213**: 427.
Head, H. (1889). Jour. Physiol., **10**: 1.
Hinsey, J. C., Ranson, S. W. and Doles, E. A. (1930). Amer. Jour. Physiol., **95**: 573.
Magnus, R. (1909). Pflüger's Arch., **130**: 219, 253.
——— (1910). Pflüger's Arch., **134**: 545, 584.
McDowall, R. J. S. (1946). Jour. Physiol., **104**: 392.
Morton, G. M., and Stavraky, G. W. (1949). Gastroenterology, **12**: 808.
Owen, A. G. W. and Sherrington, C. S. (1911). Jour. Physiol., **43**: 232.
Pi-Suner, J. and Fulton, J. F. (1929). Amer. Jour. Physiol., **88**: 453.
Pollock, L. J. and Davis, L. (1924). Arch. Neurol. and Psychiat., **12**: 288.
Sherrington, C. S. (1900). Proc. Roy. Soc., **6**: 67.
——— (1905a). Proc. Roy. Soc., **75**: 269.
——— (1905b). Proc. Roy. Soc., **76**: 160.
Sherrington, C. S. and Sowton, S. C. M. (1911a). Proc. Roy. Soc., **83B**: 435.
——— (1911b). Proc. Roy. Soc., **84B**: 201.
——— (1911c). Jour. Physiol., **42**: 383.
Socin, C. and Van Leeuwen, W. Storm (1914). Pflüger's Arch., **159**: 251.
Spadolini, I. (1948). Arch. Int. de Physiol., **55**: 317.
Stavraky, G. W. (1934). Jour. Pharmacol. and Exper. Therap., **50**: 79.
——— (1943). Trans. Roy. Soc. Can., Sect. V, **37**: 127.
Stavraky, G. W. and Graham, A. R. (1950). Federat. Proc., **9**: 317.
Teasdall, W. D. and Stavraky, G. W. (1949). Federat. Proc., **8**: 155.
Verzar, F. (1923). Pflüger's Arch., **199**: 109.
Von Uexkull, J. (1904). Zeitschr. f. Biol., **46**: 1.
Welsh, J. H. (1948). Johns Hopkins Hosp. Bull., **83**: 568.
Wyss, O. A. M. (1940). Pflüger's Arch., **243**: 457.
——— (1947). Arch. Neerl. de Physiol., **28**: 444.

MÉMOIRES DE LA SOCIÉTÉ ROYALE DU CANADA
TOME XLIV : TROISIÈME SÉRIE : JUIN 1950
CINQUIÈME SECTION

Relation entre le diagramme de solidification et la toxicité du système Diméthylamino-phényldiméthylpyrazolone–Bromodiéthylacétylurée

Par JULES LABARRE, M.S.R.C., et LOUIS TROCHU

NOS connaissances sur la synergie en thérapeutique indiquent qu'une action biologique complémentaire ou potentialisatrice se maniféste généralement lorsque des substances médicamenteuses associées s'adressent à un même système physiologique, mais par des voies différentes de pénétration dans les tissus (Bürgi, 1922).

Ce phénomène a pour conséquence une intensification de l'action biologique pour un même dosage de l'une ou de l'autre des substances considérées dans l'association. Pour peu que ces substances diffèrent entre elles dans leur processus d'élimination, la dose toxique de leur mélange devient égale ou supérieure à la somme des doses toxiques de chacune d'elles. Ainsi on peut atteindre une même intensité d'action thérapeutique avec une posologie restreinte, en accroissant conséquemment la tolérance des constituants d'un mélange.

L'action synergique résulte, du point de vue des médicaments, d'un ensemble de conditions physico-chimiques, plus ou moins faciles à préciser. Outre la structure moléculaire proprement dite, la solubilité, les propriétés électriques, le coefficient de partage (huile et eau) etc., il y a lieu de considérer dans les associations synergiques l'affinité des constituants médicamenteux entre eux, et peut-être aussi la formation de combinaisons secondaires s'opérant au cours de leur association même ou à leur passage simultané au niveau des tissus.

Dans cet ordre d'idées, les analgésiques et les uréides à chaînes ouvertes peuvent être avantageusement associés de façon à ce que l'action pharmacologique des premiers soit intensifiée par la présence des seconds, ou inversement (Niborg, 1926).

En nous appuyant sur les considérations ci-dessus, nous avons voulu vérifier sur deux produits chimiques, dont la toxicité est de même ordre et qui sont susceptibles d'une action synergique lorsqu'ils sont associés, les relations qui peuvent exister entre les propriétés thermiques de leurs mélanges d'une part et leur tolérance dans l'organisme animal d'autre part.

A cette fin, après avoir élaboré le diagramme de solidification de deux constituants, le diméthylamino-phényldiméthylpyrazolone et le bromodiéthylacétylurée, nous avons comparé la toxicité totale de quelques-uns des mélanges situés sur ce diagramme.

DIAGRAMME DE SOLIDIFICATION

Techniques. Les données numériques qui composent le diagramme de solidification ont été obtenues en opérant dans des conditions identiques de chauffage et de refroidissement, et sur des matières premières purifiées par recristallisation.

Les prises d'essai avaient un poids total de dix grammes chacune, et les composants étaient mêlés intimement au mortier, puis fondus dans un bain de toluol à reflux maintenu à l'ébullition (110° C.). A cette température, en effet, tous les mélanges étudiés entraient en fusion (sauf évidemment les substances pures) sans danger de décomposition. Le mélange ainsi fondu était ensuite porté dans un appareil à refroidissement lent et contrôlé.

Cet appareil (Fig. 1) se compose d'un tube laboratoire *c*, renfermant la prise d'essai en fusion. A l'intérieur de ce tube plonge une extrémité d'un thermo-couple, l'autre extrémité de celui-ci étant maintenue à 0° C. dans un bain de glace fondante. Un agitateur *a* mû à vitesse constante est placé également à l'intérieur du tube laboratoire. La paroi extérieure du tube est entourée d'un élément chauffant *e* qui servira au besoin à maintenir la fusion au début de l'opération.

L'assemblage ci-dessus est isolé des variations brusques de température par un double manchon *d* fermé aux deux extrémités et à l'intérieur duquel, il est possible de faire circuler au besoin un léger courant d'air refroidissant *g*. Le tube intérieur du manchon *d* est aussi entouré d'un élément chauffant *f* maintenu à une température de 60° C. Ce dispositif a permis d'accumuler des données avec une efficacité satisfaisante. La marche de refroidissement était enregistrée automatiquement à l'aide d'un appareil Speedomax.

Chaque prise d'essai a fait ainsi l'objet d'un graphique individuel présentant un point d'inflexion ou un palier à la température de cristallisation. Les variations de température étaient notées jusqu'à ce que le mélange soit complètement solidifié. Les expériences ont été répétées de deux à cinq fois pour chacune des proportions étudiées, et les données qui ont servi à la construction du diagramme définitif de solidification représentent des moyennes de lectures.

Résultats. Les températures de solidification orrescpondantes aux points d'inflexion ou aux paliers décrits sur les courbes de refroidissement de chaque mélange sont inscrites sur la figure 2. Il en résulte

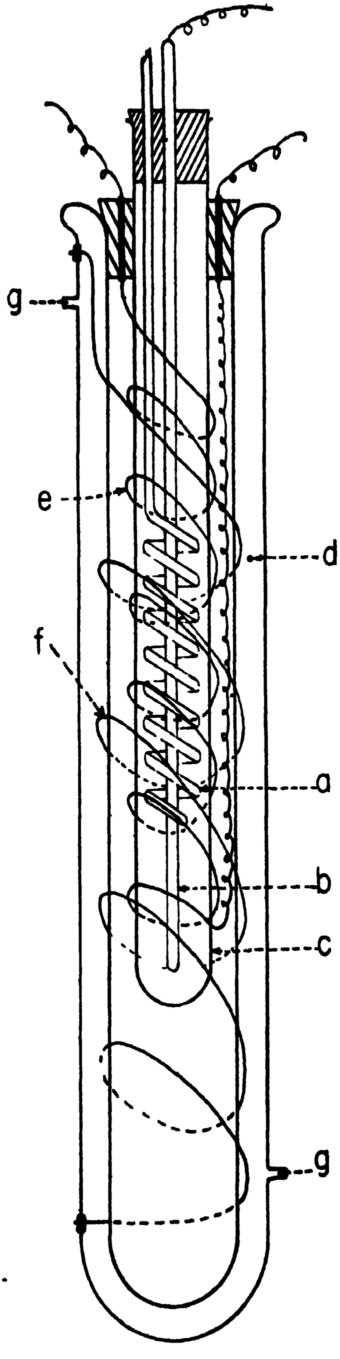


FIGURE 1.—Appareil de refroidissement du type Johnston (1928).

*un diagramme dont l'image diffère de celle d'un simple mélange ordi*naire.

L'allure descendante régulière *Aa* pour les concentrations riches en *A* (diméthylamino-phényldiméthylpyrazolone), depuis 100% à 60% environ, démontre que cette première zone ne donne lieu à aucun phénomène chimique de combinaison.

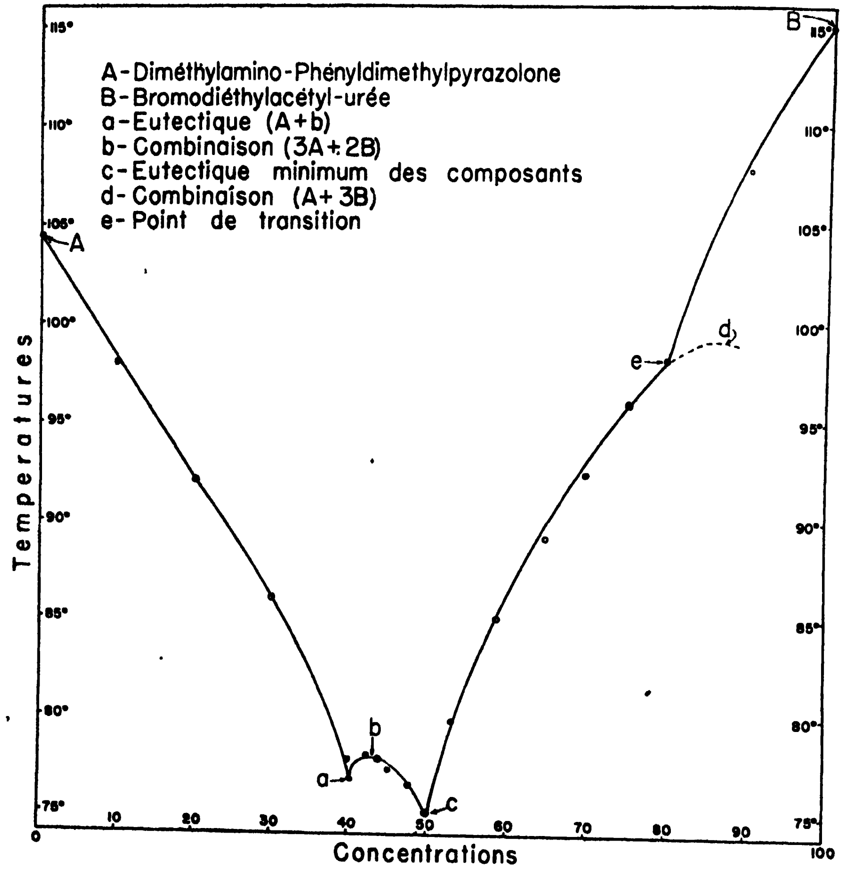


FIGURE 2. Diagramme de solidification du système binaire Diméthylamino-phényldiméthylpyrazolone–Bromodiéthylacétylurée.

La seconde partie du diagramme, comprise entre *a* et *c* décrit une courbe d'équilibre correspondante à la formation d'un composé défini. Un maximum de condensation s'effectue au point *b*, c'est-à-dire au voisinage des proportions de trois molécules de *A* et de deux molécules de *B* (Bromodiéthylacétylurée). Cette considération confirme des vues exprimées antérieurement.

Le point *a* représente ainsi les proportions d'un premier eutectique dont l'un des composants serait cette première combinaison définie.

La portion *Bc* du diagramme est caractérisée principalement par la présence du point de transition *e* et par une légère incurvation de la ligne *ec*. Ces caractéristiques semblent résulter de l'influence d'une seconde combinaison située en *d*, dont le maximum de condensation apparaît au-delà des limites du liquidus. Cette combinaison se dissocie partiellement au point *e* pour disparaître graduellement au cours du trajet *ec*. Le point *c*, à la température de solidification la plus basse, peut être considéré comme l'eutectique minimum d'un ensemble de composants.

TOXICITÉ

Techniques. Les doses toxiques de divers mélanges ont été établies sur des rats pesant environ 150 grammes. Les mélanges ont été injectés en suspension huileuse et par voie sous-cutanée. Nous avons considéré comme dose toxique L.D. 50 la quantité minimum de substance nécessaire pour amener la mort de l'animal ou plus exactement de la moitié d'un groupe de six rats dans un délai de vingt-quatre heures après l'injection.

Les mélanges de diméthylamino-phényldiméthylpyrazolone (A) et de bromodiéthylacétylurée (B) utilisés à cette fin ont été préalablement fondus. Après refroidissement, la masse cristalline était soigneusement broyée au mortier avant sa mise en suspension dans l'huile de coton.

Résultats. Les résultats des recherches de la dose mortelle L.D. 50 sont rassemblés dans la figure 3. On constate tout d'abord que les composants pris isolément sont de toxicité égale : 0.10 gramme par 100 grammes d'animal.

Les mélanges 80%A–20%B, et 20%A–80%B, qui sont situés sur le diagramme, dans des régions ne donnant lieu à aucune combinaison définie, ont une limite totale de tolérance supérieure à celle des composants purs : 0.13 gramme par 100 grammes d'animal.

Cette valeur nous indique une augmentation de la tolérance proportionnelle à la quantité du deuxième composant qui se surajoute à la dose mortelle du premier composant. L'action biologique est donc additive, et elle laisse supposer des processus différents de métabolisme et d'élimination pour le diméthylamino-phényldiméthyl pyrazolone (A) et le bromodiéthylacétylurée (B). Le point de vue ci-dessus est confirmé par la toxicité des mélanges aux proportions 65%A–35%B, et 35%A–65%B.

Cependant, l'association 50%A–50%B présente une caractéristique particulière. Sa tolérance, qui est de 0.225 gramme par 100 grammes d'animal, est nettement supérieure aux doses mortelles réunies des deux composants, soit 0.20 gramme par 100 grammes d'animal.

On se trouve vraisemblablement en présence d'une action non seulement additive, mais potentialisatrice. Il convient de noter que ce mélange correspond, sur le diagramme de solidification, à un eutectique minimum où interviennent non seulement les substances *A* et *B*, mais aussi l'influence de composés définis, illustrés par les points *b* et *d*. L'intervention de ces derniers a donc pour effet d'accroître encore davantage le degré de tolérance totale.

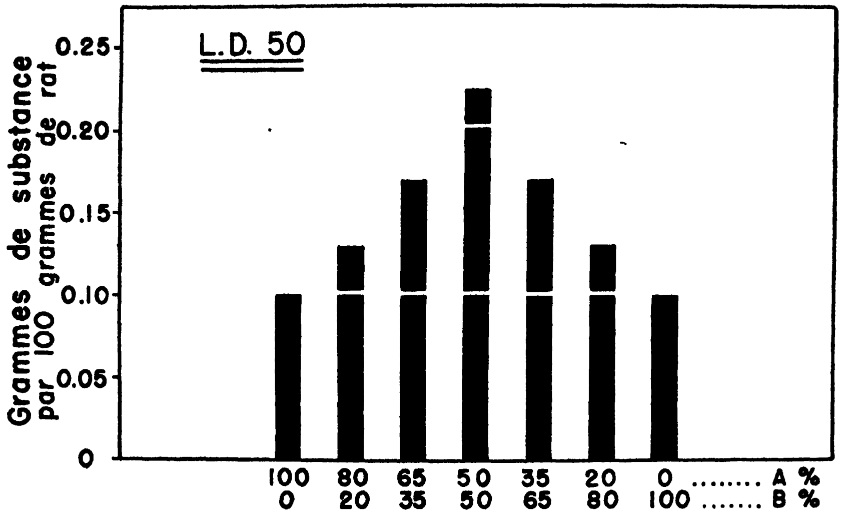


FIGURE 3.—Valeurs comparées de la tolérance (L.D. 50) chez le rat.

Il convient d'indiquer ici que les actions convulsives ou hypnotiques, provoqués par de fortes doses de diméthylamino-phényldiméthylpyrazolone ou de bromodiéthylacétylurée respectivement s'atténuent mutuellement lorsque ces produits sont injectés en association. C'est ainsi, par exemple, qu'avec le mélange 50%A-50%B, les animaux ne manifestent ni tremblement convulsif, ni hypnose très profonde. L'action correspond plutôt à une sédation profonde.

CONCLUSIONS

Le système binaire diméthylamino-phényldiméthylpyrazolone (A) et bromodiéthylacéthylurée (B) fournit un diagramme de solidification (*cooling curve*) mettant en évidence l'existence de deux combinaisons chimiques définies : l'une, stable, renfermerait 3 molécules de *A* et 2 molécules de *B*. L'autre, dissociable, 1 molécule de *A* pour 3 molécules de *B*.

Le graphique précise, de plus, deux points d'eutexie situés aux températures de 77° C. et 75° C. respectivement.

La toxicité chez le rat (L.D. 50) indique, pour quelques mélanges étudiés, une tolérance additive.

Aux proportions correspondantes à l'eutectique minimum, du diagramme, cette tolérance est non seulement additive, mais synergique potentialisatrice.

REMERCIEMENTS

Nous désirons remercier sincèrement M. Marcel Rinfret, professeur de chimie physique à l'Université de Montréal, pour ses conseils éclairés, et pour les appareils de précision qu'il a mis à notre disposition.

Nos remerciements s'adressent également à la maison Rougier Frères, ainsi qu'à l'Office de Recherches Scientifiques du Ministère de l'Industrie et du Commerce de la Province de Québec, pour l'aide technique qui nous fut accordé.

BIBLIOGRAPHIE

BÜRGI, E. (1922). Pflüger's Arch. f. d. ges. Physiol., **147**: 275.

NIBORG, S. (1926). J. de pharm. et chim., **4**: 476.

CHINOIN-SANABO (1926). Apoth.-Ztg., **74**: 1001.

JOHNSTON, J. et STEINER, L. C. (1928). J. Phys. Chem., **32**: 912.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIV : SERIES III : JUNE, 1950
SECTION FIVE

A Consideration of Muskrat Populations and Population Trends in Manitoba

By J. A. McLEOD

Presented by R. A. WARDLE, F.R.S.C.

REGARDLESS of the multitude of smaller or greater problems with which the fishery or wildlife manager is confronted almost daily, his concern mainly revolves around two over-all factors of major importance, namely, the quality and the size of the crop produced on a sustained yield basis. It is not to be denied that the quality of the crop produced merits a good deal of careful consideration but in the early stages of management studies the main energies are usually directed to a consideration of the size of the crop, the factors which are responsible for it and how they can be altered to make them more favourable.

During the past three decades much has been learned regarding the influence of weather, food, population pressure, predation, etc., on the abundance and distribution of animal species. It has also been learned that, regardless of the apparent environmental conditions, many animal populations tend to show depressions and elevations at reasonably regular intervals. Those population variations of the oscillatory type involving a simple predator-prey relationship have been explained adequately by Volterra (1926) and Gause (1935). At the present time much is known about the occurrence and periodicity of cyclic fluctuations in abundance of different animal species but, as yet, we are largely at a loss to explain their cause or causes. Certainly, attempts to correlate cyclic fluctuations with weather or food conditions have mainly been futile and the sun-spot theory which looked so promising for a time has now been discarded (MacLulich, 1937; Elton and Nicholson, 1942). Such fluctuations appear to be most pronounced in certain mammals and birds of the north temperate zone which are largely herbivorous (Rowan, 1948), but recent investigations have demonstrated that the phenomenon is exhibited by a wide range of both terrestrial and aquatic organisms of the northern hemisphere (Dymond, 1947).

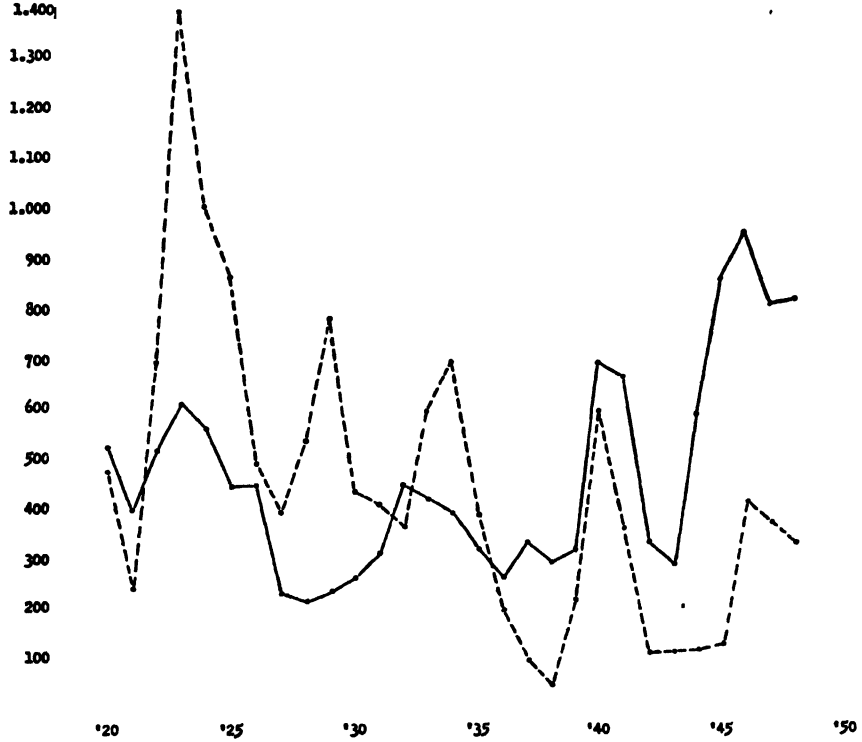
Some fifteen years ago governmental agencies undertook the management of several large potential marsh areas in Manitoba for the purpose of rehabilitating small fur-bearers such as the muskrat (*Ondatra zibethica alba*) whose habitat had been greatly reduced by drought conditions. The project has been highly successful and much of the original marsh area has been converted back into a mesosere by artificial flooding with an accompanying increase in fur production. However, in some cases the expected improvement of habitat did not follow flooding and in other cases the improvement of habitat did not result in consistently increased muskrat production in keeping with the carrying capacity of the area.

In 1947 a detailed ecological study of representative portions of several of the areas was undertaken in an effort to determine what conditions were optimum and how these conditions could best be obtained. The investigation is continuing and a considerable bulk of ecological data has been accumulated, only a small portion of which has been published (McLeod, 1948, 1949). At the outset it was assumed that, given proper protection, the muskrat population would soon exceed the carrying capacity of the area and the surplus over and above adequate breeding stock could be taken as the annual crop. It was soon discovered that no rule-of-thumb could be adopted in connection with trapping quotas because during some periods the muskrat population increased so enormously that destruction of habitat threatened. Similarly, during other periods the population remained static or even decreased in the face of adequate conditions of food and coverage and in the absence of trapping. Such happenings appear not to be peculiar to small, isolated patches but to extend over wide areas, if not over entire provinces. In an attempt to correlate muskrat abundance with environmental conditions it became necessary to take into consideration the whole matter of muskrat populations and population trends. This phase of the work has been greatly facilitated by the fact that from the outset each Fur Rehabilitation Block has been divided into Areas and Zones for administrative purposes and very accurate records have been kept of the estimated fall populations and the annual crops of pelts taken. Some of these data are reported here in the hope that they may throw some light on the riddle of population fluctuations.

POPULATION FLUCTUATIONS

Prior to 1936 no attempts were made to estimate accurately muskrat populations in any part of the province except on certain private lands and the only criterion of abundance or scarcity is the number of pelts marketed annually. The most extensive and complete records of the fur catch in Canada are those of the Fur Department of the Hudson's Bay Company. The records of the annual muskrat catches from 1820 to 1927 for the Northern Department of the Company, which covered approximately what is now western Ontario, the Prairie Provinces, and the Mackenzie River basin, were compiled and set out in graphic form by Elton and Nicholson (1942).

As pointed out by these authors, their data are not an accurate index of the population of muskrats at any time as many other factors such as market price, the number of trappers employed, and the number of pelts obtained by competitive agencies, in addition to population density, were reflected in the final figures. Similarly, the area concerned was not always the same but increased or decreased from time to time.



Graph 1.—Muskrat pelts in thousands marketed annually (1920-48) from Manitoba (solid line) and Saskatchewan (broken line). Dominion Bureau of Statistics.

A consideration of data on annual muskrat pelt returns from a comparable area gathered by the Dominion of Canada, Bureau of Statistics since 1920 shows higher over-all populations but a continuation of the same general population trends. In both cases the

muskrat pelt returns showed definite fluctua
a periodicity of about 9.5 to 10 years.

It is agreed widely that population maxima and minima are not always synchronous over a wide area since a portion of the territory may lag as much as two or three years behind the remainder. Because of this, total figures for a wide area may show the general trend but the peaks and depressions will be minimized considerably. This is illustrated by a comparison of data from the provinces of Saskatchewan and Manitoba between 1927 and 1937. However, if the data are considered to represent an interrupted single cycle in Saskatchewan, the returns from the two provinces synchronize closely and show the usual periodicity (Graph 1).

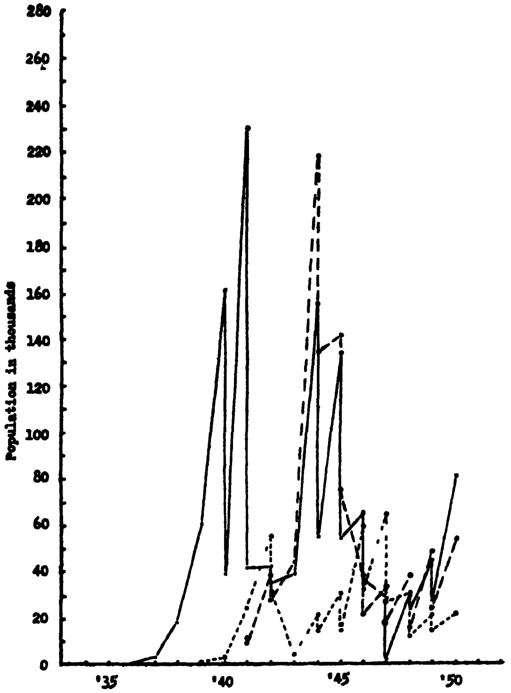
The writer (1948) presented some data which are not entirely in agreement with Graph 1 or the figures given by Elton and Nicholson. Here only small areas, locally designated as zones, were considered for purposes of population trend studies and an estimation of the annual winter population was based on a count of winter lodges rather than on pelt returns. These very limited data showed moderately synchronous fluctuations in eleven different zones at intervals of about four to five years.

MUSKRAT POPULATION GROWTH FORMS

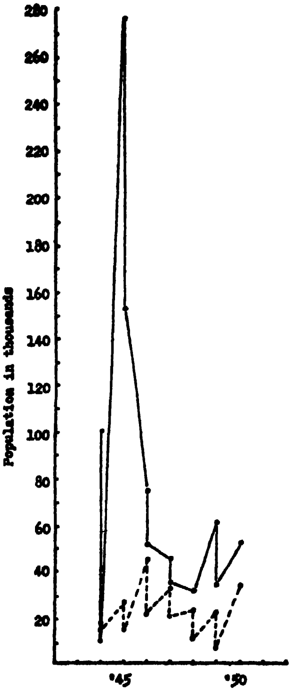
In Manitoba muskrat management has largely been directed toward the production of the largest and most adequate habitat and at the same time an attempt has been made to regulate trapping and other forms of predation so as not to deplete the breeding stock or have an excessively large population present at any time. The procedure has been to divide each area into numerous small fixed units or zones with geographic boundaries. Each autumn when lodge building had been completed, a count of winter lodges in each subdivision was made and the records compiled as described by Allan (1941) and Dozier (1948). In recent years it has been assumed that, on the average, five muskrats occupied each lodge and in this way an estimation of the population was arrived at. The trapping quota for each subdivision was then determined and unless the crop was harvested with much greater ease than anticipated the quota was not exceeded.

Such a system has operated reasonably well in the past and the data on Manitoba muskrat populations are thought to be about as accurate as those on any wild animal population. However, it has two obvious defects. First, it is almost certain that the ratio of muskrats to lodges will vary from time to time and place to place and, secondly, the system completely disregards natural mortality between the time of lodge construction and the time of trapping, an interval often as long as four and one-half months.

Graphs 2 and 3 represent attempts to show not only muskrat population trends but also the influence, if any, of trapping on the trend and on the general population level. Here entire areas within the various fur rehabilitation blocks are taken as units and the absolute data available include the estimated number of muskrats based on winter lodge counts and the number of specimens harvested each year. In these graphs the years referred to are not calendar years but crop years which terminate about May 1, after all trapping had been concluded but before the season's reproduction had begun. In this way



GRAPH 2



GRAPH 3

GRAPH 2.—Annual production of muskrats in Summerberry area (solid line), Two Island area (dotted line), and Connolly area (broken line). Upper points represent estimated fall populations based on lodge counts. Vertical lines represent the number of pelts harvested.

GRAPH 3.—Annual production of muskrats in Netley area (solid line) and Delta area (dotted line).

the reduction due to trapping can be properly matched against the previous reproductive period.

All the areas referred to in the graphs suffered at one time from a lesser or greater degree of drought under which condition they existed largely as dried out plains with occasional pot holes containing shallow water and correspondingly sparse muskrat populations. In each case within one or two years prior to the taking of the first lodge census flooding began and has continued with few or no interruptions ever since. Following the appearance of surface water there was a tremendous growth of marsh plants for from three to five years and then, providing the water level remained static or increased, the plants showed a progressive decline. Only an almost complete removal of the surface water for about two months during the summer has been found to check this decline and bring about renewed growth of the plants.

It was generally assumed that the increase in muskrat population would correspond closely with the increase in carrying capacity of the area and in the early stages of marsh management this proved to be true. Considering the population of the Summerberry area (Graph 2), for which the greatest amount of data is available, the increase appears to form a fairly typical sigmoid curve similar to that of Pearl (1930). It was also assumed that as the upper asymptote of the population curve was approached or reached heavy cropping might be indicated in order to prevent destruction of habitat and accompanying oscillations in population. At any rate, it was thought that reasonably drastic reduction of the population to the "rapid growth" phase of the curve by trapping would be compensated for quickly and that the process could be repeated annually.

Such population recoveries are shown in varying degree in the population curves of other areas shown in Graphs 2 and 3, but the best examples are those of the Summerberry area for the crop years of 1940 and 1944 and the reproduction periods immediately following. Here heavy reductions of up to 120,000 specimens were replaced by one summer's reproduction and no net loss was apparent. During other years the population shows a low rate of recovery even though trapping was limited or absent.

FACTORS INFLUENCING POPULATION CHANGES

At the outset of the present investigation in 1957 studies were undertaken on the influence of soil type, water depth, and vegetation type and density on the muskrat-carrying capacity of an area (Reeve, unpublished). However, the results to date have not been as clearly defined as anticipated and correlations between habitat size and type and muskrat abundance are not particularly evident. For example, the muskrat populations during peak years as shown in Graphs 2 and 3 approached the carrying capacities of the areas but at other times they were considerably less. It would appear that the size and quality of the habitat determine the limits of the population which can exist in it but that other factors are responsible for most of the variations within those limits.

A brief study has been made of natality, dispersion, and mortality in Manitoba muskrats in an effort to explain population changes which cannot be attributed directly to habitat conditions.

Natality. The following gives a yearly comparison of natality rates of muskrats in various Manitoba marshes:

Crop year	*Number of litters examined*	*Total number of young*	*1st litters*	*2nd litters*	*3rd litters*	*4th litters*	*Average litter size*	*Total number of young per breeding female*
1948	27	141	21	6			5.3	6.8
1949	63	447	37	16	9	1	7.1	12.0
1950	122	750	67	34	18	3	6.7	11.2

Such estimated yearly natality rates, while admittedly subject to some error, are thought to be sufficiently accurate to explain in part the variations in rate of population increase shown in Graphs 2 and 3. Providing the adult sex ratio was equal, the yearly rate of increase would be from 3.4 to 6 times and, providing the mortality rate was nil or nearly so, it would explain all the population increases excepting that shown in the Netley area during the summer of 1944. The natality rate shown for 1949 is thought to be somewhere near the maximum for Manitoba and the phenomenal estimated increase of 28 times in Netley in 1944 can only be explained on the basis of a decided underestimation of the breeding population of that spring. This is possible because a large number of bank-dwelling muskrats occur in the area and these are not included in the estimates.

Mortality. Figures on mortality rates among muskrats from trapping are quite detailed and accurate but mortality rates from other causes are known only in a general way. Mink which were once regarded as serious predators of the muskrat but whose status as such has been considerably minimized in recent years (Errington, 1943, 1946a) have been heavily trapped in the fur rehabilitation blocks and other natural enemies are of rare occurrence. Intraspecific predation is known to occur, particularly in over-abundant, insecure populations but no pronounced mortality from this cause has been noted locally. A few sporadic cases of death from malformation or from injury of unknown origin have been noted in the field but these were remarkably low for a wild animal population. Drowning, particularly of young, during severe storms has been observed on a few occasions but it is mainly confined to those in exposed situations.

Death among muskrats from known infectious disease is not common and is quite variable. On two occasions very small outbreaks of infection with *Staphylococcus aureus* were noted but the mortality was light. In addition a few suspected cases of tularemia have come to hand. However, every major decline in the past has been accompanied by an epizootic of Errington's virus enteritis (Errington, 1946b) or some similar disease. For example, in the Summerberry area during the late winter of 1941 and again in 1945 many dead specimens were found in the snow and when the ice broke large numbers of carcasses floated up. The same situation was observed in even more pronounced form in the Netley area in the spring of 1945.

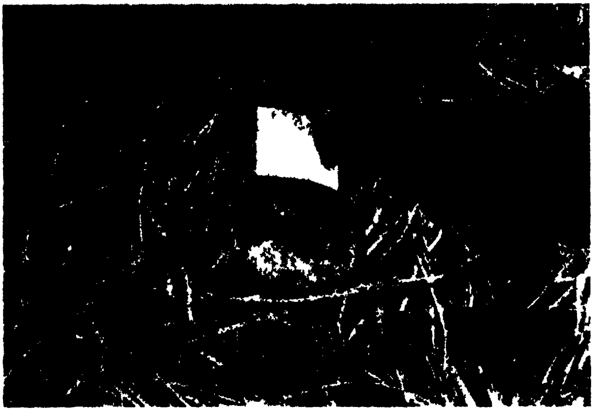


FIGURE 1.—Photograph of a female muskrat and seven young which had died of Errington's disease in Netley marsh When found the female in the foreground was in the normal nursing position on top of the brood nest which contained the young.

It was thought at one time that the epizootic disease occurred only at times of high population density but sufficient data are presently at hand to indicate strongly that it recurs at regular intervals of 4 to 5 years regardless of population density. How high a population will build up in the interval between epizootics appears to depend on a wide variety of environmental factors. The epizootic type of disease has been found locally only during the winter and early spring months but a similar disease in enzootic form has been found to persist at all times of the year and particularly during the reproductive season (Fig. 1).

Dispersion. Dispersion apparently has played only a small part in influencing trends of muskrat populations in Manitoba. It is certain that considerable random movement takes place within an area during the ice-free months and particularly during the late autumn when winter lodge construction begins. However, the amount of movement between the area and the surrounding territory is usually so small as to be negligible.

In the Netley area during the crop year of 1945 the population was very dense and some overcrowding was evident. Approximately 20,000 specimens were removed by trapping to alleviate conditions but in late autumn severe flooding from the Red River occurred and a mass migration involving thousands of muskrats took place. The general direction of migration lay to the northwest and it is reported that some specimens moved into sloughs as far as forty miles away. The following spring flooding again took place accompanied by severe ice action and another extensive migration resulted. It is estimated that the two migrations involved at least 50,000 muskrats.

CONCLUSIONS

Evidence gathered to date indicates that Manitoba muskrats tend to show cyclic fluctuations in abundance but the peaks and depressions recur at intervals of from 4 to 5 years rather than at intervals of 9.5 to 10 years. In addition, the population data from definite marshes or areas over a period of 14 years show that the fluctuations are often more pronounced than a study of the over-all pelt returns would indicate. Many of the fluctuations are synchronous in widely separated areas but the time appears to be determined by the date of initial flooding of the marsh which is invariably followed by an upswing of the population.

The carrying capacity of the marsh determines the approximate upper limits of the muskrat population which can be present but numerous other factors are responsible for variations within these limits.

The natality rate of Manitoba muskrats was found to vary from 3.4 to 6 young per adult from year to year. This would only partially explain the variations in the population growth curves shown in Graphs 2 and 3.

Dispersion has been a minor factor in connection with muskrat populations in the majority of Manitoba marshes. It does, however, partially explain the enormous decrease in the population of Netley Marsh during the crop year of 1945 (Graph 3).

Mortality in muskrats of less than three months of age has been found to be remarkably low in Manitoba with the exception of sporadic cases of enzootic disease or occasional drowning. Epizootic disease, however, appears to occur regularly at intervals of 4 to 5 years regardless of population density and usually the mortality reaches 50 per cent or higher. The epizootic frequently strikes in several marshes simultaneously but the reason for this cannot be explained clearly at present. There would appear to be some connection between the time of initial flooding and the occurrence of the first outbreak, usually with an interval of 4 or 5 years between the two. However, these outbreaks may be attributable to some general factor which is common to all the marshes of the province.

Enzootic disease has been responsible for consistently low populations in certain localized patches of marsh but it plays little part in producing cyclic fluctuations in population numbers. Within limits trapping pressure appears not to influence the general population trend excepting when extinction is approached. Epizootic disease of unknown etiology is considered to be the factor of major importance in periodically reducing muskrat populations. It is thought that the lines representing population growth rates in Graphs 2 and 3 would always show uniform upward slopes if winter mortality due to epizootic disease had been taken into account in calculating spring breeding populations.

ACKNOWLEDGMENTS

The work reported on herein was carried out under the sponsorship of the Game and Fisheries Branch, Department of Mines and Natural Resources, Province of Manitoba and the writer wishes to express his sincere thanks to members of the staff for their kind co-operation and support. Special thanks are given to Mr. G. W. Malaher, Director, for much valuable assistance and to Mr. John Melvin for the use of his records.

REFERENCES

ALLAN, D. J. (1941). New fur trails. Civil Service Rev., 14: 100-5.

DOZIER, H. L. (1948). Estimating muskrat populations by house counts. Thirteenth N. A. Wildlife Conference: 372-92.

DYMOND, J. R. (1947). Fluctuations in animal populations with special reference to those of Canada. Trans. Roy. Soc. Canada, 3rd ser., 40, sec. V: 1-34.

ELTON, CHARLES and NICHOLSON, MARY (1942). Fluctuations in the numbers of the muskrat in Canada. J. Animal Ecology, **11**: 96-126.

ERRINGTON, P. L. (1940). Natural restocking of muskrat vacant habitats. J. Wildlife Management, **4**: 173-85.

——— (1943). An analysis of mink predation on muskrats in north-central United States. Iowa Agric. Exp. Station, Res. Bull., 320: 797-924.

——— (1946a). Predation and vertebrate populations. Quart. Rev. Biol., **21**: 144-77, 221-45.

——— (1946b). Special report on muskrat disease. Quart. Rept., Iowa Cooperative Wildlife Research Unit (mimeo.).

GAUSE, G. F. (1935). Experimental demonstration of Volterra's periodic oscillation. J. Exper. Biol., **12**: 44-8.

PEARL, R. (1930). The biology of population growth. New York: Alfred A. Knopf.

MACLULICH, D. A. (1937). Fluctuations in the numbers of the varying hare (*Lepus americanus*). Univ. of Toronto Studies, Biol. Ser., no. 43: 1-136.

McLEOD, J. A. (1948). Preliminary studies on muskrat biology in Manitoba. Trans. Roy. Soc. Canada, 3rd ser., **42**, sec V: 81-95.

——— (1949). Some aspects of muskrat management in Manitoba. Thirty-ninth Convention of the Int. Assoc. of Game, Fish and Conservation Commissioners: 64-71.

ROWAN, W. (1948). The ten-year cycle. Univ. of Alberta, Dept. of Extension. Pp. 1-11.

VOLTERRA, VITO (1926). In ALLEE *et al.* (1949). Principles of animal ecology. Philadelphia and London: W. B. Saunders. Pp. 271, 372.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIV : SERIES III : JUNE, 1950
SECTION FIVE

Specific Action of Chloromycetin in the Case of Pasteurella Felis Infection in Man*

By J. ÉDOUARD MORIN, F.R.S.C.

DURING the last year, we had to deal with the case of a patient suffering from an infection seldom encountered, which was caused by a micro-organism of the Pasteurella group and upon which chloromycetin had a rapid healing action. The aim of the present communication is not only to demonstrate the specificity of this new antibiotic on a bacillus of the genus Pasteurella, but furthermore to point out the peculiar and as yet undescribed biological properties of this particular micro-organism.

The term *pasteurellosis* is ascribed to a group of infections which are usually transmitted to man by mammals, by rodents, or by birds. Some are generalized and most deadly, such as plague and fowl cholera, others are localized and of a milder evolution, such as tularemia. The micro-organisms responsible for these infections were named Pasteurella by Trevisan in 1887 after Pasteur, who isolated the prototype, *P. aviseptica* (fowl cholera), in 1880.

Case History

In the beginning of March, 1949 a wounded veteran was admitted to the hospital for chronic pain and stiffness in the right knee. The physical examination revealed a wound (Fig. 1) on the dorsal face of the left hand, in the fifth metacarpal region. In the centre were two small ragged purulent ulcerations separated by a strip of skin three millimetres wide. The surrounding area, about three and a half centimetres in diameter, was purplish and swollen. The skin adhered firmly to the under layers. According to the patient, the wound had been caused by a cat bite, a month previously. Subsequently the usual signs of infection (pain, swelling, local high temperature) had appeared. The inflammatory process, which had been suppurating from the beginning, had reached the subjacent metacarpus. The usual treatments of infections, such as local disinfection, wet dressings, sulfa drugs, and penicillin, were of no avail. We then undertook a series of bacteriological determinations (morphology, staining properties, culture growth, serology, and pathogenic activity) which led us to the diagnosis of Pasteurella felis. While these experiments were going on, the bone was surgically cleaned and streptomycin was administered. Nevertheless the infection kept progressing, and in the daily dressings were found large amounts of pus containing small gram-negative bacteria. As soon as the diagnosis of Pasteurella was well established, the patient was given, on the first three days of June, 1949, a daily dose of 3 grams of a recently discovered antibiotic, chloromycetin, which at the time was being assayed in the laboratories of Parke, Davis and Company.

---

*Contribution from the Department of Bacteriology, Faculty of Medicine, Laval University, and the Veterans' Hospital, Quebec.

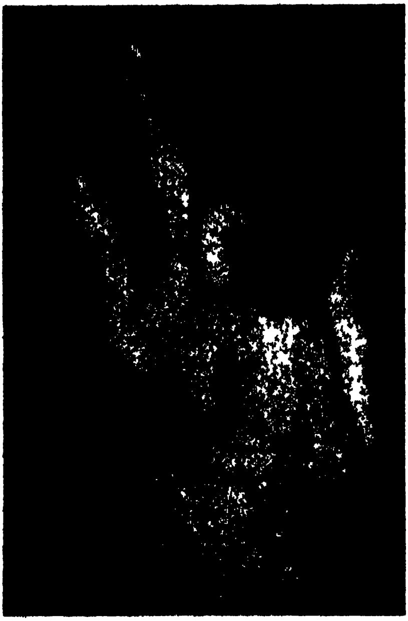


FIGURE 1 Aspect of the wound before treatment.

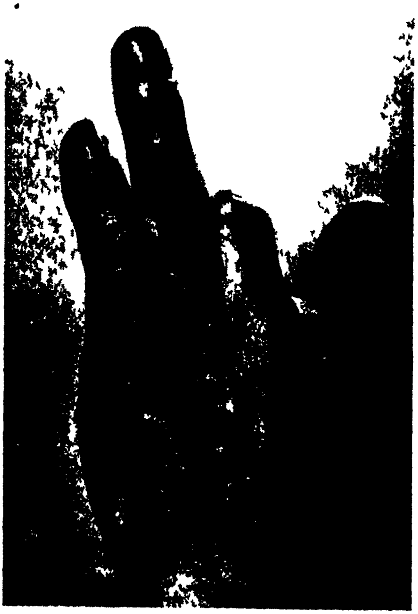


FIGURE 2 Aspect of the hand 72 hours after the third daily dose of chloromycetin

The results were spectacular. the infection, which had persisted for four months and a half, vanished in seventy-two hours (Fig 2). And, on the seventh day, the wound was definitely healed.

However, to this remarkable action of chloromycetin were to be added other peculiar data, obtained during the detailed study of the Pasteurella felis itself,

CHARACTERISTICS OF GENUS PASTEURELLA

The Pasteurellae are gram-negative, bipolar-stained, non-motile, pleomorphic, capsulated bacilli, varying in length from 0.5 to 2 5 microns. They either gather in groups or form short chains. Facultatively aerobic, their maximum growth temperature is 45° C , their minimum, 15° C , and their optimum temperature is 37° to 38° C. They slightly acidify carbohydrate media without gas formation, do not liquefy gelatin, and do not coagulate milk Such are the classical characteristics of the Pasteurellae, which at first fitted perfectly the strain that we were dealing with (Figs 3, 4, and 7)

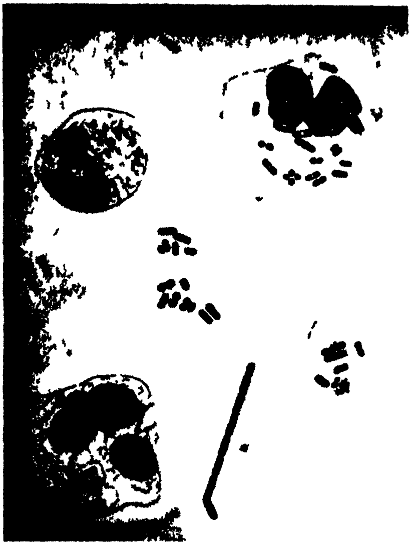


FIGURE 3 —Exsudate

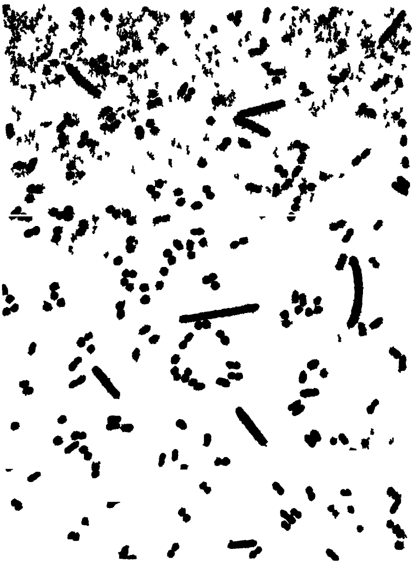


FIGURE 4 Pasteurella felis upon isolation on Lowenstein's medium

However, after repeated passages, a peculiar transformation took place in the *morphological appearance of the strain,* as it gave birth to two phases with definite differences in their shape and size We therefore undertook, with the technical assistance of Dr. Morgante, in November, 1949, a detailed study of this strain which was inoculated every second week on Francis medium and maintained at 2° C.

On chocolate agar, were obtained, after four days, two pin-head, pulvinate, circular, whitish colonies, some glistening, others dull. Under the stereoscopic microscope the surface looked granular and sugar-like. Both colonies were made of gram-negative bacilli and their pleomorphism suggested a possible contamination. Some were coccoid, others club-shaped. The protoplasm was pale, containing deep-stained, coarse, and irregular granulations. The general picture was that of Corynebacterium diphtheriae (Figs. 5 and 6). These bacilli are non-motile

Both colonies were then inoculated on the usual culture media, including rabbit-serum-carbohydrate medium. Variant "A," obtained from the glistening smooth colonies, produced no sugar fermentation, grew well on nutrient agar, abundantly on Francis medium, but not on potassium tellurite medium. Nitrates were reduced, but there was no indol or H_2S formation. Intraperitoneal inoculation of a 0.5 cc solution to a guinea pig furnished a serum with an agglutination titre of 1/640.

Dull colonies of variant "B" gave quite different results. On nutrient broth and agar growth was very poor The following carbohydrate media were acidified without gas formation (Table I).

TABLE I

Carbohydrate medium	*pH*	*Acid formation*
Arabinose	6 5	+
Dextrine	6 5	+
Dulcite	6 8	+
Galactose	6 5	+
Glucose	6 0	+
Inosite	7 0	–
Inulin	7 0	–
Lactose	7 0	–
Levulose	6 3	+
Maltose	6 0	+
Mannite	7 0	–
Raffinose	6 3	+
Saccharose	5 7	+
Salicin	7 0	–
Sorbite	7 0	–
Xylose	6 5	+

On Bordet-Gengou and chocolate agar growth was very luxuriant. As for strain A, when nitrates were reduced there was no indol or H_2S formation.

From these results it could be concluded that the original strain had split into two variants with specific differences. Furthermore, the pathogenic activity had considerably decreased.

Experiments were then repeated from a subculture of the original strain. It was lyophilized at −55° C. and kept, for six months, at

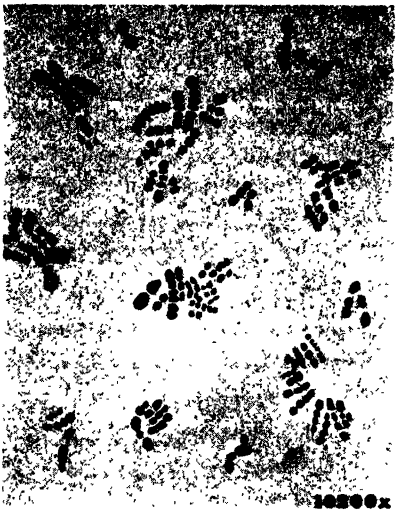


FIGURE 5.—Subculture of Pasteurella felis after six months on Francis medium.

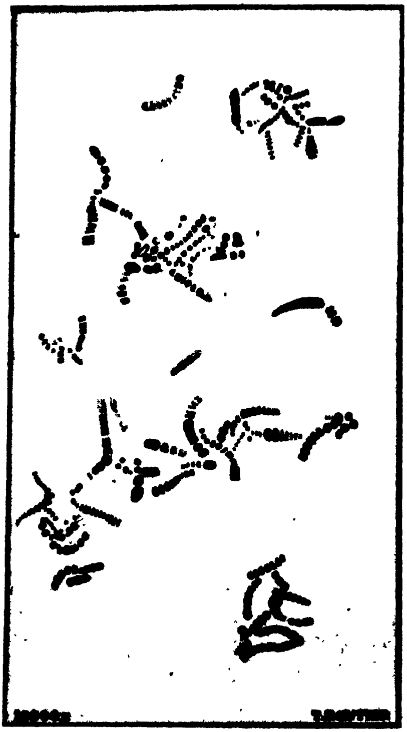


FIGURE 6.—Pasteurella felis after lyophilization.

room temperature. It was then rejuvenated on Francis medium The macroscopic and biological characteristics of the cultures obtained after five days were identical with the ones described before. Upon staining, we noticed that the bacilli, much larger in size than strains A and B (Fig. 6), were pleomorphic, club-shaped, or curved. Their protoplasm contained granulations varying in form, size, and number These granulations are deeply stained by the Neisser and Layborn methods.

Our observations led us to study several other strains of Pasteurellae, secured from the Pasteur Institute of Paris, in order to attempt mutations similar to those obtained with Pasteurella felis Preliminary observations, after three weeks, already show a beginning of mutation with the presence of protoplasmic granulations, exactly as was noticed in the felis strain. Therefore, we might conclude that Pasteurellae in general are liable to spontaneous mutations in their morphology on selective media, such as Francis medium.

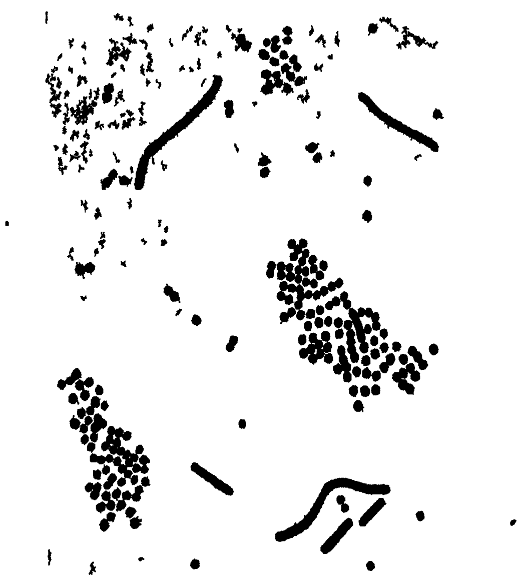


FIGURE 7 —Pasteurella aviseptica

Finally by successive passages in nutrient broth and pepton medium, we were successful in obtaining micro-organisms with the same characteristics as those of the original strain From this it is evident that Pasteurella felis is also capable of reversible morphological modifications in the proper media.

CONCLUSIONS

In concluding, we wish to point out that many aspects of our strains are still to be explored. But the results already obtained are at least sufficient to demonstrate (1) the specificity of chloromycetin on Pasteurella felis infections in man; (2) the mutational properties of the micro-organism and its dissociation in two variants with individual biological characteristics.

COROLLARY

Pasteurella felis and Pasteurella pestis being particularly alike in their biological aspects, we might presume that choloromycetin could also have a specific action on the most deadly of all the Pasteurellae, that is, plague.

It is furthermore noteworthy that a meticulous survey of the literature, up to this date, has failed to show any use of chloromycetin against plague.

REFERENCES

ALLIN, A. E. (1942). Cat bite wound infection (3 observations). Canad. M. A. J. **46**: 48.

ALLOTT, E. N. *et al.* (1944). Infection of cat-bite and dog-bite wounds with Pasteurella septica J. Path. & Bact. **56**: 411-15.

CHAMBERLAND et JOUAN (1906). Les Pasteurella. Ann. Inst. Pasteur **20**: 81.

CHAMBERS, W. E. (1942). Med. Bull. Veterans Adm. **19**: 206-7.

COLLINS, M. M. (1933). The transmission of tularemia by the domestic cat. New Orleans M. & S. J. **86**: 105.

COOPER, T. V. and MOORE, B. (1945). Pasteurella septica infection of cat-bite wound. Lancet **1**: 753-4.

HANSMANN, G. H. and TULLY, M. (1945). Cat bite and scratch wounds with consequent Pasteurella infection of man. Am. J. Clin. Path. **15**: 312.

LENORMANT, C. (1941). Les Pasteurelloses humaines par morsures de chats. Presse méd. **49**: 946. 1941.

ROSENBUSCH, C. T. and MERCHANT, I. A. (1939). A study of hemorrhagic septicemia Pasteurellae. J. Bact. **37**: 69.

RIVERS, T. M. and BAYNE-JONES, S. (1923). Influenza-like bacilli isolated from cats. J. Exper. Med. **37**: 131.

TEISSIER, P., GASTINEL, P. *et al.* (1922). Contribution à l'étude des Pasteurelloses, à propos d'un cas de Pasteurellose humaine. J. de physiol. et de path. gén. **20**: 212-41.

THAMBIDURAI, J. C. (1929). An investigation of the serological relationships of twenty-six strains of Pasteurella. J. Path. & Bact. **32**: 355.

YUSEF, H. S. (1935). Classification sérologique des Pasteurella. J. Path. & Bact. **41**: 203.

BERGEY, D. H. (1948). Manual of determinative bacteriology. Baltimore: Williams & Wilkins. 6th ed., p. 546.
DUBOS, R. J. (1948). Bacterial and mycotic infections of man. Philadelphia: Lippincott. 1st ed., p. 409.
GASTINEL, P. (1949). Précis de bactériologie médicale. Paris: Masson. 1949 ed., p. 432.
HAUDUROY, P. (1937). Dictionnaire des bactéries pathogènes. Paris: Masson. 1937 ed., p. 308.
JORDAN, E. O. and BURROWS, W. (1949). Textbook of bacteriology and immunity. Philadelphia: W. B. Saunders. 15th ed., p. 503.
TOPLEY, W. W. C., WILSON, G. S., and MILES, A. A. (1947). Principles of bacteriology and immunity. London: Edward Arnold. 3rd ed., p. 767.
VERGE, J. (1947-8). Cours de microbiologie. Paris: Institut Pasteur. P. 143.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLIV : SERIES III : JUNE, 1950
SECTION FIVE

Variations in the Silk-Spinning and Locomotor Activities of Larvae of the Spruce Budworm, *Choristoneura fumiferana* (Clem.), at Different Rates of Evaporation[1]

By W. G. WELLINGTON

Presented by J. R. DYMOND, F.R.S.C.

THE investigation reported here developed during a study of the temperature and moisture reactions of the spruce budworm, *Choristoneura fumiferana* (Clem.). Aggregations and responses of larvae in combined gradients of temperature and moisture have been described (Wellington, 1949a; 1949b). It was demonstrated that larvae aggregated primarily in response to rates of evaporation, and that the aggregations formed at rates of evaporation peculiar to each instar.

Numerous laboratory investigations of the preferences which various animals exhibit in gradients of physical factors have been reported, and the existence of preferences has generally been interpreted to imply ecological importance of the phenomenon. Such studies should include some demonstration of the relation of observed preferences to the requirements of the animals in their natural habitats. Since the completion of the experimental programme cited above, considerable laboratory and field data on the relation of weather factors to behaviour of the spruce budworm have been accumulated, so that some information on the role of preferred zones in the ecology of the insect is now at hand. Some of the more recent findings are reported herein.

MATERIALS AND METHODS

1. *General Considerations*

From the standpoint of dispersal, establishment, and survival, the first, second, and sixth instars have proved to be particularly important periods in the larval life of the spruce budworm. Consequently, larvae of these instars were chosen for further laboratory study. Since previous work demonstrated the importance of rate of evaporation to the larvae, this phenomenon was investigated more fully. An activity important in the field behaviour of larvae of each of the instars was selected and its function was investigated at different, constant rates of evaporation. Apparatus previously described (Wellington, 1949a) was used to demonstrate simple aggregations in gradients; methods used in the experiments reported here are described below. At this point a comment on the notations used for the presentation of evaporation data is necessary, although the system is presented more fully in the references previously cited.

---

[1] Contribution no. 2660, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.

It has been demonstrated (Wellington, 1949b) that, in moisture gradients, aggregations which were stable enough to be of any value for inter-instar comparisons were obtained only after pre-conditioning larvae in saturated air. When such aggregations were considered as frequency distributions, plotted against rates of evaporation, the regions of stable aggregations proved to be those of the modes. For each instar, the narrow range of evaporation peculiar to the region of the mode was designated the instar rate. The instar rates of present interest are as follows: instar I, 0.09–0.10 cu. mm. per min.; instar II, 0.13–0.14 cu. mm. per min.; instar VI, 0.04 cu. mm. per min.

All measurements of evaporation were made with the micro-evaporimeter previously described (Wellington, 1949a). Admittedly, the presentation of these rates as decimal fractions of a cubic millimetre is somewhat confusing to those more familiar with the notations of relative humidity and saturation deficiency. Nevertheless, the method is retained for reasons elaborated in the earlier paper, which may be summarized by pointing out that, of the three notations, only rates of evaporation are stable values over ranges of temperature. For instance, in terms of per cent relative humidity, the range of evaporation rates noted for the first instar extends from 48 to 62 per cent between 15 and 27° C. Similarly, the rates for instar II range from 22 to 55.5 per cent between 15 and 32° C., and those for instar VI range from 73 to 78 per cent between 15 and 36° C. The objection to per cent relative humidity may be applied also to saturation deficiency, since this notation also depends upon air temperature (Leighly, 1937; Thornthwaite, 1940).

2. *Experimental Methods*

Instar I. The first-instar larvae spin hibernacula without feeding. Observations of larvae in gradients indicated that the maximum aggregation occurred at the rate of evaporation at which most larvae began to spin. Since successful spinning is essential for survival, the ability to spin was investigated further at different constant rates of evaporation.

Constant rates ranging from 0.00 (saturation) to 0.16 cu. mm. per min. (*circa* 36 per cent R.H. at 20.6° C.) were established in a series of sealed jars by means of sacks of silica gel and blotter squares moistened with drops of water. Jars were kept at 20.6° C. throughout the period of observation.

Four hundred newly emerged larvae were introduced into each jar. These were observed at the intervals noted below until 50 per cent of a group had begun to spin. The beginning of silk-spinning was chosen as the check point instead of the end of spinning, because the latter point was frequently difficult to determine. Intervals for observations were as follows: every 10 minutes for the first eight hours, and every 20 minutes from the eighth to the eighteenth hour. Thereafter, checks were made every 6 hours until the thirtieth hour, and then every 5 hours until the fiftieth hour. As an added precaution, groups held at the limits of the range were checked periodically for several days. These observations were made over a period of time whenever sufficient larvae became available. Six thousand eight hundred larvae were used. The results are presented graphically in Fig. 1.

Instar II. Under natural conditions the second-instar larva emerges from its hibernaculum in the early spring and moves to the outer part of a branch, where it penetrates a needle or a bud. In gradients of moisture in the laboratory, larvae were observed to move incessantly, and there was some indication that the rate of movement varied with rate of evaporation. Therefore, since the rate of crawling of the larvae is intimately linked with their ability to establish themselves in the field, variations in this rate under varying moisture conditions were investigated.

Unlike first-instar larvae, which aggregate at their instar rate without special treatment when they emerge from eggs, larvae of the second instar, because of some desiccation during overwintering, require at least four hours' acclimation at saturation (Wellington, 1949b) before the true instar rate can be demonstrated. During this conditioning period, temperature is not so critical as moisture, but, since the work reported here was done at 20.6° C., this temperature was used in the storage of larvae of this and the following instar.

Larvae were collected as they emerged from hibernacula and, when they were conditioned as noted, their rates of crawling were measured at different constant rates of evaporation. The apparatus used consisted of a condenser tube containing a cardboard platform which provided a suitable surface for crawling. The ends of the tube

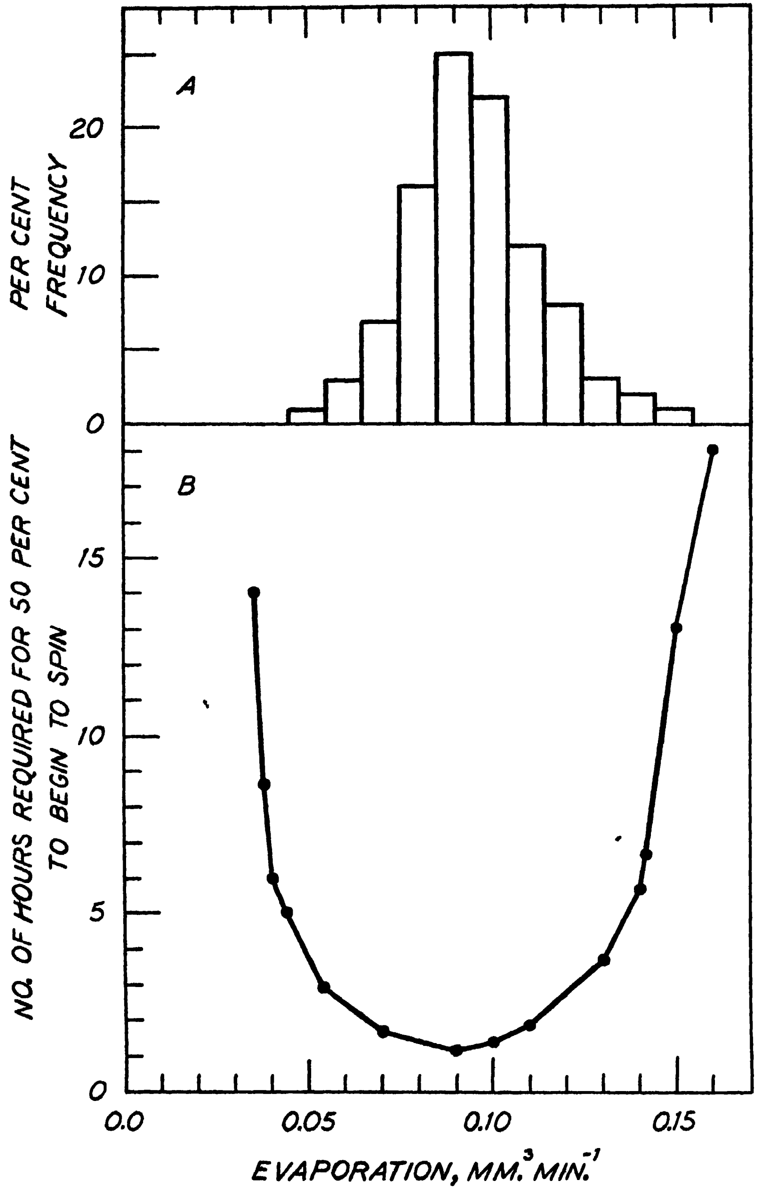


Figure 1.—The relation of the time required for first-instar larvae to begin construction of hibernacula at different constant rates of evaporation to the rates of evaporation at which larvae aggregate in a moisture gradient. *A*: Frequency distribution of larvae in a gradient of evaporation. *B*: Times required for 50 per cent of a group of larvae to begin to spin at constant rates of evaporation.

were closed with corks, one of which contained a thermocouple and the other a micro-evaporimeter. Moisture levels were obtained with moist blotters or varying amounts of silica gel. The temperature was controlled by circulating water through the jacket. Directed larval movements were obtained by using the rigidly photopositive behaviour characteristic of the instar (Wellington, 1948). One hundred larvae were used to obtain an average value for the rate of crawling at each moisture level. One thousand four hundred larvae were used. The results are shown in Fig. 2.

Instar VI. In the field the sixth instar is the period of heaviest feeding. Sixth-instar larvae construct webs or tunnels by tying together racks of needles on shoots, and they tend to remain at such feeding sites unless disturbed by external factors or by exhaustion of the food supply (Wellington and Henson, 1947; Wellington, 1948). In gradients of evaporation in the laboratory, these larvae did not move about so readily as did those of earlier instars. Consequently, since the factors causing disturbance of feeding larvae in the field were known (Wellington and Henson, 1947; Wellington, 1948) and since the amount of feeding by the larvae is linked with the amount of movement, observations were made on the amount of movement over a range of moisture conditions.

Nine hundred and fifty larvae were conditioned and placed in groups of 50 in the series of jars. Observations were made on the number moving in each jar 15, 30, 45, and 60 minutes after the beginning of the experiment. The number in motion at each constant rate of evaporation was recorded and expressed as a percentage of the total number in each group. The results are shown in Fig. 3.

RESULTS

Each of the three figures consists of two parts. For each instar the part labelled *A* illustrates the aggregation of 100 larvae in a gradient of evaporation. The part of each figure labelled *B* illustrates the results of the series of observations outlined above. These are plotted against the same scale of evaporation rates for comparison with the gradient aggregations. Details of the *B* series appear below.

Instar I. Fig. 1*B* shows the times required for 50 per cent of each group to begin spinning at given constant rates of evaporation. A comparison of this diagram with Fig. 1*A* shows that, even when no gradient of evaporation was available, the 50 per cent point was reached more quickly within the preferred zone. Under dry conditions spinning did occur, but at moisture levels greater than those of the preferendum the situation was complex. Below a rate of evaporation of 0.035 cu. mm. per min. (*circa* 81 per cent R.H. at 20.6° C.),

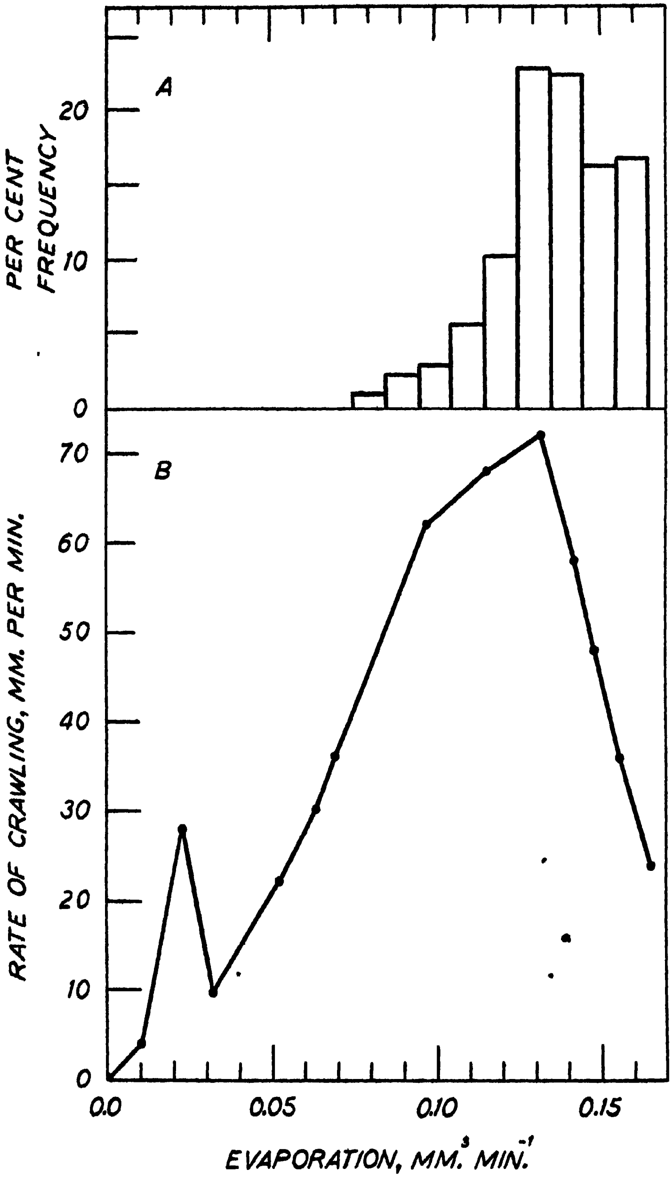


FIGURE 2.—The relation of the rates of crawling of larvae of the second instar at different constant rates of evaporation to the rates of evaporation at which larvae aggregate in a moisture gradient. *A*: Frequency distribution of larvae in a gradient of evaporation. *B*: Larval speeds, in mm. per min., at different constant rates of evaporation.

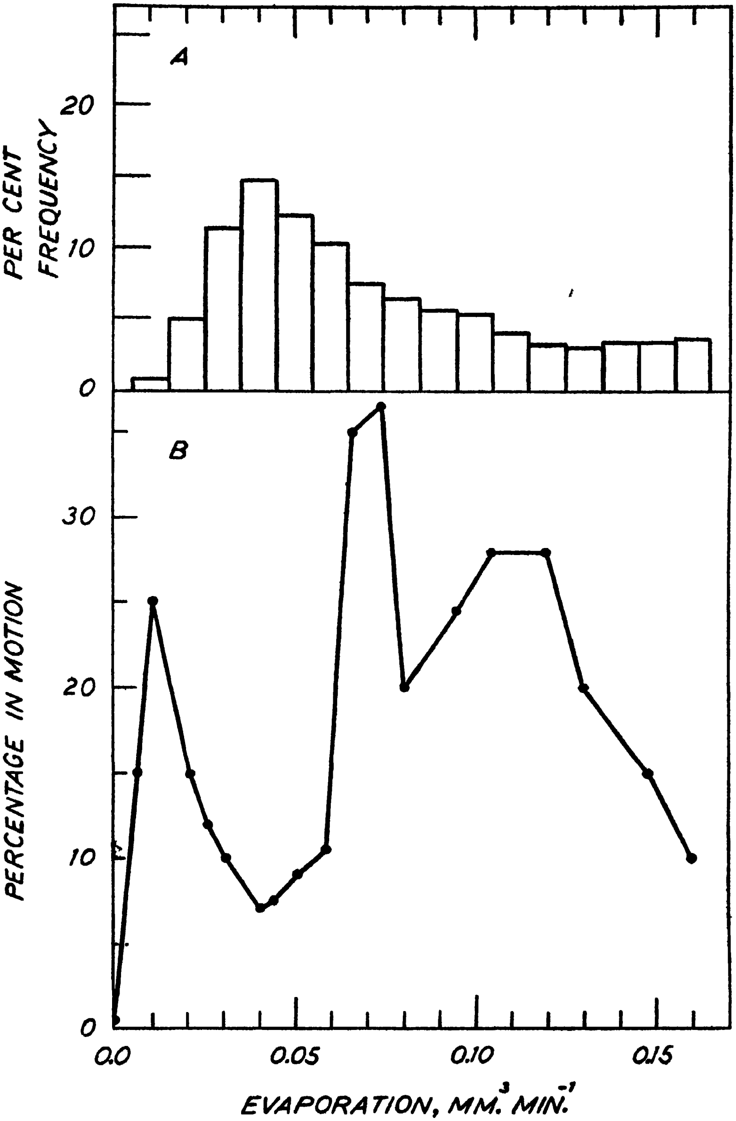


FIGURE 3 – The relation of amount of movement of sixth-instar larvae at different constant rates of evaporation to the rates of evaporation at which larvae aggregate in a moisture gradient. *A*: Frequency distribution of larvae in a gradient of evaporation. *B*: Percentages of a group of larvae in motion at constant rates of evaporation.

less than 50 per cent of a group began to spin. Fig. 1*A* shows that, in a gradient where other conditions were available, larvae did not move to this zone. At constant rates lower than this the percentage of the population which could begin to spin decreased rapidly, until, at or near saturation, less than one larva in 1,000 was able to begin construction of a hibernaculum. This is one aspect of a general observation that spruce budworm larvae of all instars are effectively immobilized if they are kept constantly in saturated air. Although larvae appear capable of moving under these conditions, ordinarily they do not. First-instar larvae have been kept at saturation for two weeks without spinning. At room temperatures, the mortality rate increases sharply after this time; but, when larvae are placed at 0° C. in saturated air, they may be kept for at least three months. Spinning occurs when they are transferred to warmer, drier conditions.

Instar II. A comment on the frequency distribution shown in Fig. 2*A* is necessary. This distribution is somewhat distorted at the higher rates because, in the apparatus used, rates above 0.16 cu. mm. per min. could not be obtained. When not obstructed, some larvae are able to move to zones where rates are as high as 0.18, so that their presence in such a limited gradient distorts the distribution. Nevertheless, the range of evaporation defining the preferendum is clearly marked.

Fig. 2*B* shows the average rate of crawling, expressed in mm. per min., of larvae exposed to different constant rates of evaporation. The preferred zone is indicated by the more rapid rates of crawling. Above and below the zone there is a rapid decline in the speed of movement.

The secondary peak near 0.02 cu. mm. per min. (83.5 per cent R.H. at 20.6° C.) was not spurious. It was of extremely constant occurrence. Near saturation there was a definite temporary increase in speed, which dropped off rapidly at lower rates of evaporation. This appeared to be an interesting manifestation of the general avoiding reaction which may be observed when paths of larvae are traced in gradients of moisture illuminated by diffuse light (Wellington, 1949b). In such gradients sharp turns appear in the paths when they touch extreme conditions. At constant rates of evaporation the reaction takes the form illustrated in Fig. 2*B* when movement is directed by light.

Once again, saturation produced effective immobility. At saturation larvae could be stored for two weeks at room temperature, or for two months near 0° C.

Instar VI. Fig. 3*B* shows the percentage of each group in motion at the various constant rates of evaporation. Apart from immobilization at saturation, the amount of activity was least within the preference zone (cf. Fig. 3*A*). Fig. 3*B* is of particular interest because it shows distinct peaks on either side of the preferendum. The peak near saturation is still another manifestation of the characteristic specific reaction to air approaching saturation. The peak at higher evaporative rates (comparable to 63–68 per cent R.H. at 20.6° C.) is a response to drier conditions, which may be explained by reference to activity under natural conditions. This point is amplified in the discussion.

DISCUSSION

Previous experiments demonstrated the manner in which larvae of the spruce budworm tended to aggregate when atmospheric moisture was made available in gradient form. The results of this investigation have shown the extremely close relationship between these aggregations, considered in terms of rates of evaporation, and the manner in which certain activities are performed at constant rates of evaporation. Field observations show some of the relations of the preferred zones to spruce budworm ecology.

It was shown that larvae of the first instar spin most rapidly at the preferendum. No field evidence exists to corroborate this observation; although it has been observed that no spinning takes place at very low rates of evaporation (Henson, 1950), very high rates have not been recorded in the field during the period of first-instar activity. Therefore, observation of the range of evaporation under field conditions is still incomplete for the present purpose.

Information on the second instar is more adequate. When larvae are moving from hibernacula to feeding sites, spring polar air masses may sometimes provide evaporative rates close to those inducing maximal crawling rates. Such high rates of evaporation may occur in the larval habitat even when measured atmospheric rates of evaporation are comparatively low. For instance, in a description of conditions linked with larval drift, which requires initial movement along the branches, Henson (1950) has reported air temperatures averaging 19.2° C. and relative humidities averaging 71.7 per cent in the ecoclimate of the stand. These values would give evaporative rates centred around 0.049 cu. mm. per min., a value far below the instar rate. Nevertheless, it has been shown that, with clear or partly cloudy skies associated with larval wandering (Wellington and Henson, 1946), solar heating of exposed coniferous foliage may raise surface temperatures at least to, and frequently higher than, 23.8° C. (Wellington, 1950). Larvae on branches are influenced by conditions within this microclimatic zone, not by those of the ecoclimate. Therefore, it is necessary to recalculate evaporation at this higher temperature. In the spring, before the beginning of transpiration, it may be assumed that the amount of water vapour in the air surrounding the old foliage is close to the amount in the lower layers of the air mass. Therefore, evaporation from a moist surface in the boundary layer should be affected principally by the increased temperature. The temperature of 23.8° C. gives a rate of evaporation of 0.112 cu. mm. per min., which is close to the preferred rate. When air movement is taken into consideration, a further increase in the rate of evaporation may be postulated.

The secondary peak in crawling speed near saturation is of particular interest when it is considered in relation to moisture conditions at the surface of an opening bud. Over such surfaces the water vapour level is higher than in the air surrounding the old foliage when the second-instar larvae are wandering. It is possible that the increased rate of movement near saturation may permit larvae to penetrate bud scales much more actively than they could otherwise.

The decreased amount of movement characteristic of the sixth-instar larvae within their preferred zone appears to be associated with normal feeding conditions. Measurements of physical conditions within the larval tunnels have been made for brief periods under natural conditions (Wellington, 1950). Even under dry external conditions (e.g., 0.112 cu. mm. per min., or 57.6 per cent R.H. at 28.6° C.), the average rate of evaporation in freshly constructed tunnels in which little feeding has occurred is 0 039 cu. mm. per min., which is, for practical purposes, equivalent to the instar rate, 0.04. On the other hand, in tunnels in which much feeding has occurred, so that outside air penetrates the gaps in the walls, the average rate is 0.079, which is near the range of the activity peak above the preferred zone. Under field conditions, the significance of this peak of activity is that larvae which have consumed most of their food supply are placed in a much more active state and are ready to respond to stimuli, such as movements of the twigs by wind, which may initiate movements to new feeding sites. Within their preferendum, larvae are so sluggish that they do not move about readily. In the field, this state prevails when they are surrounded by fresh food, and may be advantageous. On the other hand, in some laboratory rearing programmes, it may be an adverse factor. One of the chief difficulties in attempts to rear large larvae in jars is that they do not move spontaneously to new supplies of food but simply remain on the fragments of the old supply. The moisture level in closed jars is high, and it is apparent that the larvae may not be stimulated to move when the food is exhausted, for the environment is not dry enough. This can be confirmed by rearing them on twigs kept exposed to ordinary air movements. When this is done, it is found that they move readily to new shoots when the old ones are stripped.

To some extent, the tunnels of sixth-instar larvae also act as insulators against rising external moisture, but they are less effective than in maintaining a high level against dry outside conditions. Larvae kept constantly at saturation in a container are effectively immobilized, but it has also been observed that larvae in the field drop from their twigs when the tunnels are flooded during sudden showers. These observations appear antagonistic, but Fig. 3*B* shows that the marked increase in activity just above actual saturation helps to explain the anomaly. If the tunnels are flooded early enough during the shower, so that the enclosed air has not had time to reach actual saturation through simple vapour exchange, larvae will be at a higher level of activity than they are at the ordinary preference level. Accordingly, when the tunnels are flooded, the larvae react violently to the liquid water and drop from the twigs.

The diagrams illustrating rates of crawling and amounts of movement at different constant rates of evaporation provide further evidence of mechanisms which are involved in producing aggregations of larvae in gradients. Previous observations (Wellington, 1949b) showed that klinokinesis (Gunn *et al.*, 1937) was an important part of the behaviour mechanism producing aggregations of second-instar larvae. Despite the increased speed at the instar rate, an increased amount of turning kept the majority of the larvae in the aggregation zone. Now, the experiments at constant rates of evaporation have shown one aspect of a reaction which, despite the lack of a gradient, should be termed orthokinesis. This enters into control of behaviour on the moist side of the aggregation zone. A similar state has been shown to exist on either side of the sixth-instar rate. Moreover, since larvae of this instar remain quiet much of the time they are in the aggregation zone, it appears that orthokinesis is of more importance in the establishment of their aggregations in gradients than it is in the establishment of those of instar II.

Attempts have been made to define the general significance of a preferendum. For instance, it has been suggested that temperatures resulting in the lowest expenditure of energy are preferred (Zenyakin, 1938). Certain results (e.g., Vilardebo, 1948) support this conclusion.

Indeed, if the sixth instar of the spruce budworm were considered to the exclusion of the others tested, the results would conform. However, as the activity patterns of the first- and second-instar larvae are at variance with the suggestion, no generalizations on the significance of a preferendum to an organism should be made until a great deal more information is available. More extensions of laboratory results to field conditions are required.

SUMMARY

1 Spruce budworm larvae of the first instar maintained at constant rates of evaporation begin to spin hibernacula sooner within their evaporation preferendum than outside it.

2. Some larvae are able to spin at high rates of evaporation, but the percentage able to spin at rates near saturation is negligible.

3. Larvae of instar II placed at constant rates of evaporation move most rapidly at rates within their preferendum. They have also an orthokinetic response to conditions close to saturation which takes the form of a sudden, temporary increase in rate of crawling. In gradients of moisture, this is associated with a turning reaction.

4. Larvae of instar VI at constant rates of evaporation move about much less within their preferred zone than at any rates outside it, except at saturation. At rates just above and just below the zone, larvae are especially active. This excessive activity diminishes rapidly as rates of evaporation farther from the preferred zone are encountered.

5. Sixth-instar larvae in the field inhabit natural feeding tunnels in which the rate of evaporation is normally almost identical to that of the preferred zone. When these tunnels are partially destroyed, the rate of evaporation rises and the larvae become active enough to leave them. Conversely, if tunnels are flooded suddenly, larvae are activated by rates of evaporation close to saturation and react by dropping.

REFERENCES

GUNN, D. L., KENNEDY, J. S., and PIELOU, D. P. (1937). Classification of taxes and kineses. Nature **140**: 1064.

HENSON, W. R. (1950). The means of dispersal of the spruce budworm, *Choristoneura fumiferana* (Clem.). Ph.D. thesis, Yale University.

LEIGHLY, J. (1937). A note on evaporation. Ecology **18**: 180-98.

THORNTHWAITE, C. W. (1940). Atmospheric moisture in relation to ecological problems. Ecology **21**: 17-28.

VILARDEBO, A. (1948). Sur le preferendum thermique des insectes. III. Sur l'évaluation de l'activité du Grillon (*Gryllulus domesticus* L.) en fonction de la température, à l'aide du thermoactographe. Physiol. Comp. et Oecol **1**: 86-8.

WELLINGTON, W. G. (1948). The light reactions of the spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae). Canad. Ent. **80:** 56-82A.

——— (1949a). The effects of temperature and moisture upon the behaviour of the spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae). I. The relative importance of graded temperatures and rates of evaporation in producing aggregations of larvae. Sci. Agr. **29:** 201-15.

——— (1949b). The effects of temperature and moisture upon the behaviour of the spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae). II. The responses of larvae to gradients of evaporation. Sci. Agr. **29:** 216-29.

——— (1950). Effects of radiation on the temperatures of insectan habitats. Sci. Agr. **30:** 209-34.

WELLINGTON, W. G. and HENSON, W. R. (1946). The effects of variable microclimatic conditions upon the behaviour and activity of the spruce budworm. Ann. Tech. Rept., Sault Ste Marie Forest Insect Lab. **2:** 167-213 (unpublished).

——— (1947). Notes on the effects of physical factors on the spruce budworm, *Choristoneura fumiferana* (Clem.). Canad. Ent. **79:** 168-70, 195.

ZENYAKIN, L. A. (1938). Zur Frage uber den Zusammenhang der thermischen Preferenz mit der Temperaturreaktion des Gasaustauches bei *Operophtera brumata* L. und *Chloridea obsoleta* F. (Lepid.). Rev. Ent. URSS **27:** 174-80.

TRANSACTIONS OF

THE ROYAL SOCIETY
OF CANADA

SECTION V
BIOLOGICAL SCIENCES

THIRD SERIES—VOLUME XLV—SECTION V

JUNE, 1951

OTTAWA
THE ROYAL SOCIETY OF CANADA
1951

TABLE OF CONTENTS

On the Transverse Forces in a British Columbia Inlet.
By W. M. Cameron 1

On the Migration of Pacific Salmon (Oncorhynchus).
By W. A. Clemens, F.R.S.C. 9

A Comparative Study of the Myology of the Head Region in Three Species of Thamnophis (Reptilia, Ophidia).
By I. McT. Cowan, F.R.S.C., and W. B. M. Hick . . 19

Resources of Arctic and Subarctic Seas.
By M. J. Dunbar 61

The Action of Sodium Salicylate in Protein Dispersion.
By A. G. McCalla 69

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLV : SERIES III : JUNE, 1951
SECTION FIVE

On the Transverse Forces in a British Columbia Inlet*

W. M. CAMERON
Presented by W. A. CLEMENS, F.R.S.C.

THE investigation of circulation in estuaries is receiving continually increasing attention. A recent colloquium reported by Stommel (1950) has emphasized the deficiencies in the present knowledge of the dynamics of estuarine flow.

The applicability of the gradient current equation to the study of large-scale ocean currents is indicated by the widespread use of charts of dynamic topography. It has been found that directions and magnitudes of currents can be calculated accurately by assuming a balance between pressure gradients and the Coriolis force. The assumptions of a quasi-steady state, and the small magnitude of inertial and frictional forces appear justifiable.

Estuaries represent areas where the foregoing simplifying assumptions are not applicable. The flood and ebb of the tides influence the direction and magnitude of estuarine currents. Even without tidal complications, the seaward reduction of the freshwater concentration establishes large pressure gradients in the direction of mean flow. Such gradients are accompanied by accelerations and frictional forces associated with mixing of fresh and salt water. The bottom and lateral boundaries further complicate the dynamic picture. In shallow estuaries the bottom may introduce an important frictional effect and greatly influence the rate and degree of turbulent mixing.

The inlets along the coast of British Columbia represent an estuarine type in which certain of these complicating factors are suppressed. They are long, narrow, and deep. Their side-walls plunge quickly to depths approaching four hundred fathoms. They extend far inland, and relatively large streams representing extensive drainage areas empty into their heads.

The large ratio of length to width results in tidal currents which are predominately up or down inlet. Superimposed on these is the longitudinal circulation established by the run-off. As Tully (1949) has shown, the fresh water moves seaward in a shallow brackish zone with increasing velocity, mixing with salt water which moves in from the sea at greater depths.

---

*Presented Wednesday, June 6, in the Oceanographic Session arranged by the Canadian Committee on Oceanography.

The longitudinal circulation involving tidal accelerations and pressure forces established by tidal oscillations of the sea surface is complicated by the inertial forces associated with the accelerating upper zone, by the pressure field deriving from the freshwater concentration, and by eddy frictional forces. It is unlikely, therefore, that a formal solution of the equation of motion along the length of an inlet in a transient state will be forthcoming in the immediate future. However, there is evidence to suggest that the equation describing the cross-inlet components of forces is more tractable.

TRANSPORT OUT OF PORTLAND INLET

A study of the oceanography of Chatham Sound was initiated by the Pacific Oceanographic Group in the summer of 1948. Wide variations in the salinity of the surface waters of the Sound were shown to be associated with the run-off discharging into it from the Nass and Skeena rivers. Below a shallow brackish layer, the salinity increased rapidly to a depth of about sixty feet, increasing relatively slowly at greater depths.

To follow the distribution of the water from the rivers, the mean salinity at sixty feet was arbitrarily selected as that of undiluted sea-water. The deficiency from this value was interpreted as representing the dilution due to river discharge. To each salinity in the surface layer could be assigned a freshwater concentration, and by integration of the salinity profile, the average freshwater concentration above any level could be calculated.

The distribution of freshwater concentration in Chatham Sound has been presented for conditions of high and low river discharge (Cameron, 1948). The concentration decreased with relative distance from the rivers. Superimposed upon the seaward decrease of fresh-water content, there appeared evidence of variation across the line of mean flow, a higher concentration lying to the right of the direction of the current. This tendency was particularly striking at the outlet of Portland Canal, the deep inlet through which the Nass River water discharged into the sound.

Stations 30 and 31 (Fig. 1) situated at the north and south sides of the outlet close to the 100-fathom contour, were occupied during three surveys of the sound. Each survey was designed to occupy a station on four successive days at different stages of the tide. Suc-

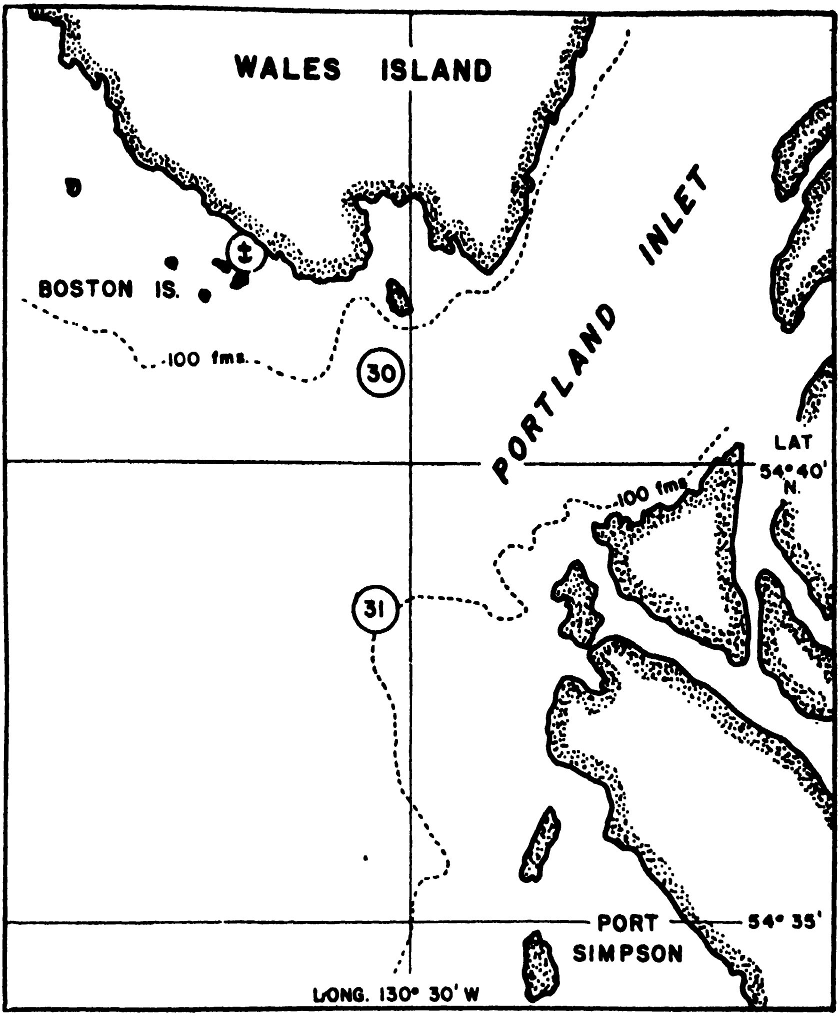


FIGURE 1.—Hydrographic and anchor stations in the vicinity of Portland Inlet, 1948.

cessive stations were occupied within forty minutes. In each of the twelve occupations, station 30 (to the north and to the right of a seaward current in the canal) showed a higher freshwater concentration than did station 31. Typical salinity profiles are illustrated in Fig. 2.

Following the method demonstrated by Jakhelln (1936), the surface velocity, the volume transport, and the freshwater transport between these stations were calculated. It was assumed that there was gradient flow at right angles to the plane through the stations, that there was a linear gradient of freshwater concentration along each pressure surface between stations, and that the isosaline pressure surface lying at approximately ninety feet represented a surface of no motion. Observations and calculations were in English units and are presented in this form in Table I.

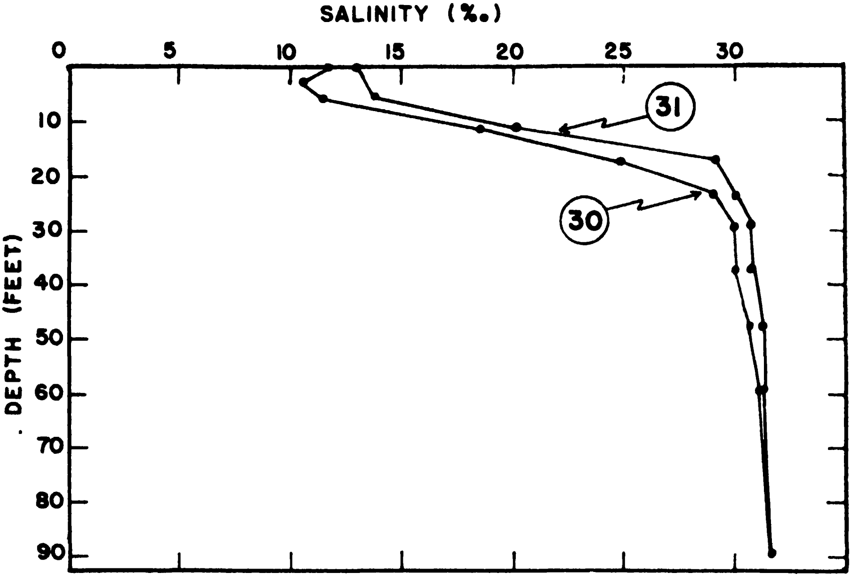


FIGURE 2.—Salinity profiles at stations 30 and 31, Chatham Sound, June 4, 1948.

Freshwater transports are plotted with the Nass River discharge data in Fig. 3. The latter are from gauge readings at Aiyansh, forty-five miles upstream from the river mouth. These data have been supplied by the Dominion Water and Power Bureau. Provision of these observations prior to formal publication by the Bureau is gratefully acknowledged. Readings for the month of July, during which no completed surveys were undertaken, have been omitted from the figure.

The calculated and observed freshwater discharges agree reasonably well. Relatively large variations are indicated in the June calculations, reflecting unsteady conditions following the peak of the run-off. The August calculations are slightly lower, on the average, than the Bureau data. In view of the several assumptions made, particu-

TABLE I

SEAWARD SURFACE VELOCITIES, VOLUME, AND FRESHWATER TRANSPORTS OUT OF PORTLAND INLET
(Calculated from dynamic height data at stations 30 and 31, Chatham Sound, assuming no motion at 90 feet)

Date	*Surface velocity* (knots)	*Volume transport* (cubic feet per second)	*Freshwater transport* (cubic feet per second)
June 1	0.45	500,000	172,000
2	0.12	251,000	73,500
3	0.21	604,000	126,000
4	0.61	336,000	117,800
8	0.48	284,000	75,000
9	0.98	570,000	130,000
10	0.17	185,000	22,300
11	0.96	384,000	107,000
Aug. 16	0.17	366,000	35,200
17	0.40	450,000	40,500
18	0.34	207,000	22,000
19	0.44	647,000	48,500

larly in respect to the distribution of fresh water between stations, the disagreement is not considered significant.

The table indicates that the decrease in freshwater transport during the summer was not accompanied by a decrease in volume transport above the assumed level of no motion. It was accomplished by a reduction in the mean freshwater concentration in the section. The large volume transports in the surface layers indicate correspondingly large up-inlet transport of the deeper saline water, the net seaward transport equalling the discharge of the river. If gradient flow exists at greater depths, the assumption of a very slight pressure gradient at ninety feet would provide for a sufficiently large up-inlet flow without seriously affecting the transports calculated above. This results from the relatively shallow depth of the freshwater layer compared to the total depth of the inlet (over 300 fathoms).

OBSERVATION OF CURRENT VELOCITIES

Data are available to indicate the reliability of the calculated surface currents. On August 31, the survey vessel anchored between Boston Islands and Wales Island at the position indicated in Fig. 1.

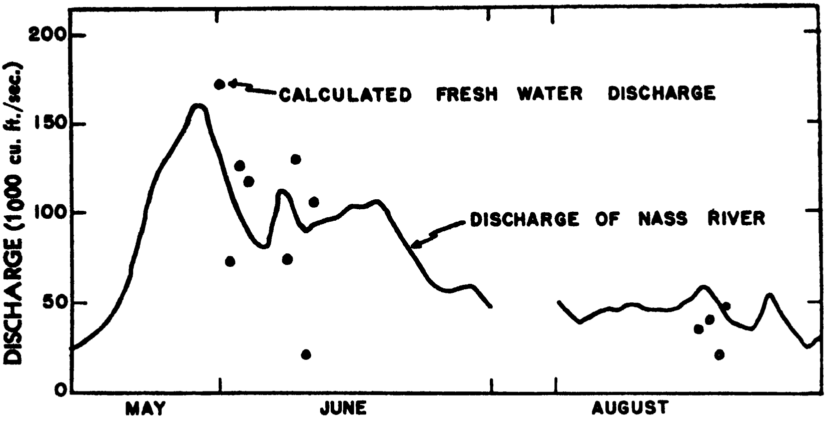


FIGURE 3.—Discharge of Nass River. Solid line from gauge data, circles from dynamic height data at stations 30 and 31.

Observations of the drift of current drags from the anchored ship are presented as current velocities in Fig. 4. The seaward components through the channel were presumed representative of the down-inlet currents at the mouth of Portland Inlet.

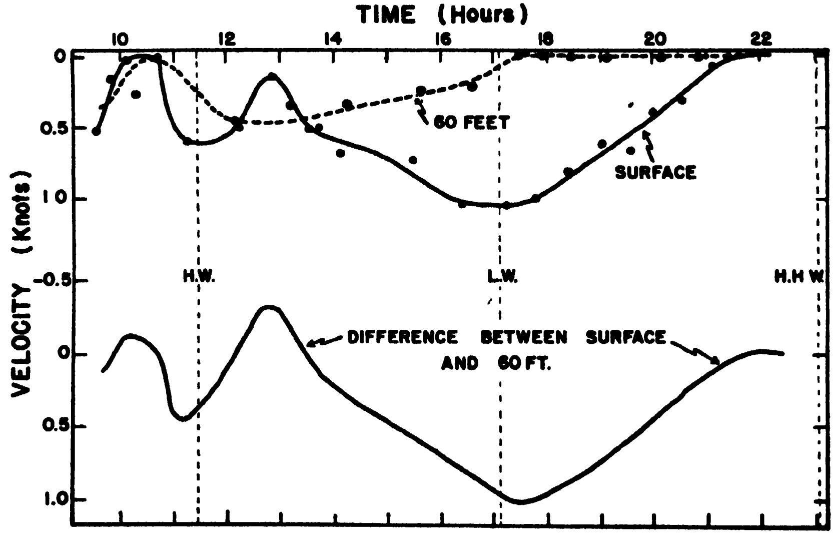


FIGURE 4.—Seaward velocities at anchor station, August 31, 1948.

The surface current was seaward over the period of observation, fluctuating in speed near high water, reaching a maximum at low water and decreasing to zero at higher high water. Its seaward velocity averaged from high to low water was 0.68 knots. The current at sixty feet was less, and differed in phase. The average difference in velocity between the surface and sixty feet was 0.34 knots.

The currents calculated from the August survey were lower than those measured at the anchor station at the end of the month. Calculated differences between the surface and sixty feet were 0.08, 0.32, 0.34, and 0.29 for the four occupations. Recalling that the calculated currents represent a mean between the two stations, the agreement of direction and approximate magnitude is satisfactory.

LACK OF PERIODICITY IN GEOPOTENTIAL SLOPE

The apparent balance between the Coriolis force associated with the net seaward flow of surface water, and the geopotential slope of the pressure surfaces occurs only in the average. It is to be expected that the velocity of the current in the surface layers of the inlet would demonstrate a periodicity determined by that of the tide, a net seaward surface velocity being required by the influx of river water at the head. No similar periodicity can be demonstrated in the data representing geopotential slopes.

This lack of agreement cannot be interpreted as indicating the presence of significant transverse inertial or frictional forces. The difference in dynamic height indicated by the profiles in Fig. 2 is determined largely by a two- to four-foot difference in level of isosaline surfaces. Internal waves of comparable amplitude and of periods of the order of one hour would seriously complicate the interpretation of dynamic height data.

If the periodicity in the dynamic height data has not been masked by internal waves, we are led to the conclusion that in the transient state there must be present either cross-inlet inertial or frictional forces or both. The apparent balance of Coriolis and pressure forces in the average suggests that the inertial and frictional forces are eliminated in the averaging process. If this is the case the frictional forces acting across the inlet must be non-dissipative or negligible.

The relatively narrow and constant width of British Columbia inlets would appear important in restricting the amplitude of the variation of inertial forces. Let us presume that at one stage of the tide there is a balance between the Coriolis and pressure forces. If the direction of the surface water reverses, these two forces would act together to accelerate the surface water in the direction of the transverse pressure gradient. Transverse movement would be restricted by the narrow width of the inlet and the vertical stability of the water. A comparatively rapid reversal of the pressure gradient would result from the accumulation of fresh water against the opposite lateral boundary, transverse movement would be suppressed, and the Coriolis and pressure forces would tend to regain their balance. Though the inertial forces may be comparatively large at any one time, the associated accelerations would act at very low velocities and the transverse movement of individual water particles would be correspondingly small. If cross-inlet velocities are low, there seems little evidence for the existence of high transverse velocity shear, and hence of significant transverse frictional forces.

CONCLUSION

Data have been presented to indicate that, on the average, there is an approximate balance between the Coriolis force and transverse pressure gradients at the outlet of Portland Canal. Since this region is similar to many British Columbia inlets, it is reasonable to infer that these are the important transverse forces in inlets of this type.

The longitudinal forces are considerably more complicated and present oceanographic problems which are common to estuary circulations in general. Considerable data are available regarding the longitudinal and vertical variation of salinity in inlets, but velocity data are sparse. Current observations in deep inlets present mechanical difficulties. If the mean velocity profiles in an inlet can be deduced from transverse dynamic sections, the co-ordinated study of mass and velocity fields can be accelerated. Investigation of this possibility is under way at the present time.

REFERENCES

Cameron, W. M. (1948). Freshwater in Chatham Sound. Prog. Rep. Pac. Fish Res. Bd. Can., no. 76: 72-5.

Jakhelln, A. (1936). The water transport of gradient currents. Geofysiske Publikasjoner, 11, no. 11: 1-14.

Stommel, H. (1950). Proceedings of the colloquium on the flushing of estuaries, Cambridge, Mass., Sept. 7-8, 1950. Woods Hole, Mass.: Woods Hole Oceanographic Institution.

Tully, J. P. (1949). Oceanography and prediction of pulp mill pollution in Alberni Inlet. Bull. 83, Fish. Res. Bd. Can.

PROCEEDINGS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLV : SERIES III : JUNE, 1951
SECTION FIVE

On the Migration of Pacific Salmon (*Oncorhynchus*)

W. A. CLEMENS, F.R.S.C.

DURING the summer of 1950, while conducting a course on the biology of fishes at the Oceanographic Laboratories of the University of Washington (located at Friday Harbour, Washington), an opportunity was presented for obtaining some information on salmon. The observations have served to confirm certain conclusions arrived at over a considerable period of years in regard to salmon migration and to the development of a picture of the phenomenon of Pacific salmon migration. While the sequence of events and the main factors involved seem to be reasonably clear in broad perspective, considerable speculation is necessary as to details. It is hoped that the following brief presentation will serve in some measure to clarify the problem and to stimulate research.

The account will deal particularly with the sockeye salmon, *Oncorhynchus nerka*, in relation to the Fraser River. The following is a review of the life history of this species. Large numbers of sexually maturing sockeye salmon enter Juan de Fuca Strait during the summer and pass through the channels among the San Juan Islands into Georgia Strait. Considerable numbers of sockeye also enter Georgia Strait through Johnstone Strait to the north. In due course the fish enter the Fraser River; they swim up the river, pass into tributaries on which there are lakes, and eventually proceed up streams flowing into the lakes, spawn, and die. The young fish emerge from the gravel in the following spring, drop downstream into the lake where they usually remain a year, and then go to sea. A few young fish may go to sea as fry and a considerable number may remain for two years in the lake before proceeding seaward. The above account applies to the majority of the sockeye populations but there are some exceptions. Spawning may take place to a considerable extent along the shores of lakes where there are seepages, and in some instances in the outlet immediately below a lake, and more rarely in a stream on which there is no lake. Undoubtedly the bulk of young fish go to the open sea by the southern route through Juan de

Fuca Strait. Possibly some go through Johnstone Strait to the north but no definite record is as yet available. The fish usually live in the open ocean for two full years and commence their freshwater migration in the fourth summer. Some, mostly males, may return in the third summer and are commonly known as grilse; some may remain an additional year and return in the fifth summer.

In dealing with the problem of migration, it is necessary to consider the behaviour pattern of the species and its relation to the physical environment. On the one hand, each species of fish has a behaviour pattern which, during the life cycle, may be modified in some respects in association with a series of physiological changes. On the other hand, the environment has an annual cycle of changes. The behaviour of the fish from time of hatching to death is the result of a series of interactions between the living organism with its cycle of physiological changes and the dynamic environment with its cyclic changes. The importance of this approach to the study of fish behaviour has been demonstrated particularly by the work of Huntsman (1945) on Atlantic salmon and of Hoar (1951) on the young of Pacific salmon.

As stated previously, the sockeye deposit their eggs in the autumn usually in the gravel of streams tributary to lakes. In the early spring free-swimming fry emerge from the gravel and are carried downstream into the lake. Undoubtedly this displacement is the result of the inability of the young fish to orient themselves or hold position in the current. During the summer the young fish tend to occupy the region of the thermocline. In the late autumn and winter, when the water temperature is nearly uniform from surface to bottom, they may have a more extensive vertical distribution. With the onset of spring the temperature in the upper waters rises, and the fish, which are now yearlings, become very active, enter the upper warmer waters and form dense schools which move about the lake. This movement, along with the steady flow of water toward the outlet, eventually brings the fish to the outlet where they are caught in the strong current and carried into the stream. The behaviour of the yearlings, including the pronounced activity, may have its cause in the increased activity of the thyroid gland, which has been shown to occur in Atlantic salmon (*Salmo salar*) (Hoar, 1939), sockeye yearlings (Hoar and Bell, 1950), and the eel (*Anguilla anguilla*) (Fontaine, 1943). Hoar states that young salmonoids show increased thyroid activity in the spring and suggests that light and the pituitary gland are involved in the physiological changes that occur in the fish.

Foerster (1937) has shown that, in Cultus Lake, migration takes place when there has been a rise in surface temperature of the lake to approximately 4.5° to 5.5° C. He also shows that when temperatures go above 10° C. migration ceases. In Cultus Lake, and in fact in all lakes studied, some young fish fail to migrate as yearlings. Evidently they retreat to deeper water as the surface water warms and fail to come under the influence of the out-going current. Ward (1932) refers to this upper warm layer as a "temperature blanket." The young fish which fail to get out of the lake as yearlings usually migrate the following spring as two-year-olds. However, some never do leave the lake. These are almost entirely males which usually mature at three years of age; they have been called "residual" sockeye by Ricker (1938). It is probable that there is a change in the endocrine complex associated with gonad development so that the reaction pattern of the residual fish may be quite different from that of the yearlings and of such a character that it does not lead them to the lake outlet.

Once in the outlet stream, the sockeye yearlings are under the influence of the current. The movement downstream is largely the result of losing position at night but when large numbers are in the stream considerable displacement may occur even in the daytime.

On reaching Georgia Strait, the young fish are still under the influence of the water current. It is well established that the set of the current from the Fraser River is northwestward in a counter-clockwise movement which turns southward with the main flow toward Vancouver Island and through Haro Strait. The current is greatly influenced by the strong tidal flows. In brief, the flood tide entering through Juan de Fuca Strait sets most strongly toward the mainland of Washington and into Rosario Strait. This flow contributes greatly to the counter-clockwise movement of the water in Georgia Strait and to the stronger outflow through Haro Strait on the ebb tide.

The strong tidal flows through the narrow passages among the islands between Juan de Fuca and Georgia Straits result in much turbulence so that the Fraser and ocean waters are well mixed. Some mixing, of course, takes place in Georgia Strait by wind action. However, the upper waters have a lower salinity than do the deeper waters (Lucas and Hutchinson, 1927).

When the young salmon reach Georgia Strait they tend to remain in the upper, less saline water. This places them in the location of the strongest seaward current, which is undoubtedly the major physical factor in the movement of the young salmon to the open sea. The current is produced primarily by the large volume of water poured into Georgia Strait by the Fraser River at this time. The discharge reaches a monthly mean of over 250,000 cu. ft. per second in June as compared with about 27,000 cu. ft. per second in March. As stated previously, the Fraser River water tends to leave Georgia Strait through the channels to the westward, chiefly through Haro Strait. During the period of spring tides and maximum Fraser River flow, velocities of five knots or more may be attained.

May, June, and July are the months in which the major outward movement of young salmon takes place. Observations on young fish show that they tend to head into a current when in a position to see objects along shore or bottom—that is, they are positively rheotactic. When the current is less than their cruising or swimming ability, they hold position or swim into the current. When the current is beyond their cruising or swimming ability, they lose position and are carried with the current; they may even swim with it. Furthermore, at night, when they cannot orient themselves by sight or by contact with the bottom, they are carried with the current. It is evident, then, that any small pelagic fish in the strong currents among the channels at the southern or northern ends of Georgia Strait will tend to be carried outward to the open sea. There would seem to be little doubt that young herring (*Clupea pallasii*) are moved outward as are the young salmon.

Sooner or later large numbers of the young salmon being carried southward from Georgia Strait are brought into the shore waters of the islands to the south, and of Vancouver Island as well. During the summer of 1950, from July 3 to August 8, large numbers of young pink salmon (*O. gorbuscha*) and considerable numbers of young chum (*O. keta*) and spring salmon (*O. tshawytscha*) were taken in beach seines along the shores of the San Juan Islands. For the most part the net hauls were made at or near low water slack and from 10 A.M. to 3 P.M. There was some indication that the young salmon were more abundant in Haro than in Rosario Strait but proof cannot be offered since the hauls were not made on comparable tides or in equal numbers. The young fish were actively feeding and their stomachs contained large numbers of small crustaceans and midge larvae and pupae (*Spanistoma pacifica*).

The numbers of fish taken in early August were definitely fewer than in July and it is presumed that the main body of fish had been carried southward into Juan de Fuca Strait prior to August.

No yearling sockeye were taken in the seine hauls. Possibly they had already passed through the channels or perhaps, being of larger size, they were farther offshore or in deeper water.

Once the fish arrive in Juan de Fuca Strait their transport to the open sea is facilitated by two circumstances: (1) during the period of heavy land drainage, which occurs in June, the outflow current is predominent in the upper waters and reaches a very high velocity at the height of the ebb tide; (2) the young fish tend to occupy the upper waters and are therefore almost continuously under the influence of the outgoing current.

The outflow from Juan de Fuca Strait moves northwestward along the Vancouver Island coast owing to the effect of the hydraulic head from the Fraser River water. The flow is opposed by the prevailing northwest winds and off Cape Beale it turns seaward and joins the California current moving southward. There is a similar flow from Barkley Sound, and all other sounds along the coast, so that there is a more or less continuous series of northward currents along the coast. This coastal counter-current system is associated with the current of the oceanic west wind by a series of eddies or partial eddies in which there is considerable upwelling of deep oceanic water (Tully, 1942). The young salmon are undoubtedly carried in the coastal current for some time. There can be no question of the northwestward movement for observations of fishermen indicate definitely that adult sockeye enter Juan de Fuca Strait from the northwest. A similar distribution apparently occurs in the cases of spring and coho salmon. Tagging of troll-caught individuals off the west coast of Vancouver Island and the Queen Charlotte Islands has demonstrated a southeastward movement of maturing fish; there must, therefore, have been an earlier northwestward movement of the younger fish.

While the tagging has clearly established that spring salmon travel long distances from the stream of origin, practically nothing is known of the distances travelled by sockeye since they are not taken commercially in the open sea. It is possible that they may have a much less extensive distribution and remain relatively near the river estuaries, though always to the northwestward because of the nature of the water circulation. The various populations of sockeye salmon from the major river systems may therefore remain as more or less discrete units, occupying fairly well defined feeding grounds and each returning to its stream of origin at a given time.

Knowledge of the factors involved in the return of the adult fish to the streams is very limited at the present time but certain facts are evident.

On the one hand, it would seem that current is the primary external or environmental factor. There is no evidence that fish can navigate in the open waters. In any case, data on temperature, salinity, oxygen, and other factors off the west coast of Vancouver Island fail to indicate any gradients which the fish could follow and travel southeastward to the entrance of Juan de Fuca Strait. The fish apparently travel well to shoreward as shown by the trolling operations for spring and coho salmon. There is no evidence that salmon appear normally in the offshore California current. The most acceptable conclusion appears to be that the fish respond rheotactically to the northwestward-flowing coastal current. Accurate knowledge of the distribution and movement of the fish must await investigation.

On the other hand, the major internal factor must be the maturation of the gonads. The development of the reproductive organs involves the endocrine complex. For example, it is well known that the pituitary is associated with the reproductive cycle. Fontaine, Lachiver, Leloup, and Olivereau (1948) have shown that increased thyroid activity is associated with the maturation of the gonads of Atlantic salmon. Hoar has similar histological evidence as yet unpublished.

Gonad development also involves utilization of the fat stores which were built up during the two or more years of vigorous feeding in the ocean. Any increase in muscular activity on the part of the fish, possibly associated with thyroid activity, will further draw upon the fat reserves. Hoar and Dorchester (1949) have shown that the utilization of fat in the goldfish (*Carassius auratus*) results in an increase in the water content of the fish's body. Undoubtedly there is a change in the osmoregulatory mechanism.

It is pertinent to recall that it has been shown that certain members of the families of Salmonidae and Clupeidae have large numbers of kidney glomeruli and therefore are equipped to cope with a low-salinity or freshwater environment (Nash, 1931). Apparently all members of the Salmonidae and Clupeidae have a well-developed system of glomeruli, distal convoluted tubules, and a patent neck segment, all of which are characteristics of freshwater teleosts (Edwards, 1935). A further point which may be of significance is the fact that both sockeye salmon and Pacific herring cease feeding or almost so with the onset of sexual maturity.

It is apparent then that the development of the gonads is accompanied by (1) a change in endocrine activity; (2) a change in body metabolism; and (3) a change in the osmotic regulation. In this osmoregulatory disturbance or change it is evident that the fish will, through kinetic orientation, tend to occupy water of lower salinity which will be surfaceward as well as shoreward because of land drainage. At the same time, they will be led to the rivers by a rheotactic response.

It may be pointed out that the migration of the Pacific herring is similar to that outlined for sockeye salmon. As already stated, undoubtedly the young herring resulting from the spawnings in Georgia Strait are carried out through Juan de Fuca Strait as are the young salmon. It is known that the herring feed and grow in the region off the entrance to Juan de Fuca Strait and each fall large numbers of maturing fish enter the Strait, pass into Georgia Strait, and eventually spawn along the shores of Vancouver Island and of the numerous islands lying eastward to it. The response of the herring is evidently identical with that of the salmon and it may be postulated that with the maturing of the gonads, there is a change in the osmoregulatory mechanism and a kinetic orientation in relation to water of lower salinity.

The salmon upon entering Juan de Fuca Strait tend to follow a route along the south shore of Vancouver Island, thence along the south shore of San Juan and Lopez islands, into Rosario Strait, and northward into Georgia Strait and to the mouth of the Fraser River. While the majority of the fish travel through Rosario Strait, considerable numbers go through the other channels among the San Juan Islands. That the movement of the fish is not always continuous toward the mouth of the Fraser, but is influenced by the strength of the tidal flow, is demonstrated by the operation of reef nets used among the San Juan Islands. These are traps with two diverging leads. They are frequently set close to shore along the islands or just within the southern point of a bay with the openings to the leads facing the ebb tide. It is evident that these shoreward fish reverse their direction of movement and travel with the strong ebb current. The seine boat operators are also aware of a back-and-forth movement of the off-shore fish with the tides. But with the greater flow of the flood tide in the channels and the active swimming of the fish, the schools eventually reach the mouth of the Fraser River. Response to the current takes the fish into the river and upstream.

The most difficult problem is to suggest an explanation for the return of sockeye salmon to the area where they lived as young. The fact that they do return with probably few exceptions has been demonstrated by many marking experiments and by knowledge of certain populations, such as that of the Adams River, tributary to the Fraser River. It may be largely a matter of timing and distance travelled, with responses to chemical substances in the water and to differences in temperatures also being involved.[1] The solution of the problem will come in due course with diligent research on the physiology of the fish and close studies of the characteristics of the environment.

---

[1] In recent paper Hasler and Wisby postulate that salmon return to their "home" stream of olfaction, having been conditioned to the water during their sojourn while young.

As forecast at the outset of this paper, major attention has been given to the movements of sockeye salmon but a few statements may be added in regard to the other species of Pacific salmon. Chum and pink salmon usually spawn in streams at relatively short distances from the sea. The young upon emerging from the gravel are carried downstream and seaward. Coho salmon spawn in the streams usually without passing through lakes and the young remain in the stream for a year before going to sea. Hoar (1951) has shown that young coho salmon are less strongly phototactic and are less responsive to current than the young pink and chum salmon. They consequently do not come into the strongest currents but hold position, occupy territory, chase intruders. and feed actively. They thus escape being carried to sea until the second spring. Spring salmon may go to sea in the first spring or the second.

In regard to the return of adults from the ocean, it would seem that the sockeye and some spring salmon respond to the freshwater influence resulting from the summer floods which follow the melting snows of the mountains. The pink, chum, coho and some spring salmon react to the freshwater resulting from the autumn rains.

The migration of Pacific salmon is another excellent illustration of the delicate interrelations between organisms and the environment; in other words, of the interplay between a physico-chemical organism and a physico-chemical environment. It is a demonstration of the adjustment of an organism through a series of physiological changes and responses to a series of environments and environmental changes. The cyclic nature of the organismal and environmental changes results in the recurring migration pattern.

The author is greatly indebted to Dr. W. S. Hoar and Dr. J. P. Tully for much helpful information and data.

REFERENCES

EDWARDS, J. G. (1935). The epithelium of the renal tubules in bony fish. Anat. Rec., **63**: 263-79.

FONTAINE, M. and CALLAMAND, O. (1943). Les aspects physiologiques d'une "vie cyclique de l'anguille d'Europe" *Anguilla anguilla* L. Bull. Mus. Hist. Nat., **15** (5): 373-8.

FONTAINE, M., LACHIVER, F., LELOUP, J., et OLIVEREAU, M. (1948). La fonction thyroidiene du saumon (*Salmo salar* L.) au cours de sa migration reproductrice. J. Physiol. (Paris), **40**: 182-4.

FOERSTER, R. E. (1937). The relation of temperature to the seaward migration of young sockeye salmon (*Oncorhynchus nerka*). J. Biol. Bd. Can., **3** (5): 421-38.

HASLER, A. D. and WISBY, W. J. (1951). Discrimination of stream odors by fishes and its relation to parent stream behaviour. Amer. Nat., **85**: 223–38.

HOAR, W. S. (1939). The thyroid gland of the Atlantic salmon. J. Morphol., **65** (2): 257-95.

——— (1951). The behaviour of chum, pink, and coho salmon in relation to their seaward migration. J. Fish. Res. Bd. Can., **8** (4): 241–63.

HOAR, W. S. and BELL, G. M. (1950). The thyroid gland in relation to the seaward migration of Pacific salmon. Can. J. Res., **D28**: 126-36.

HOAR, W. S. and DORCHESTER, J. E. C. (1949). The effect of dietary fat on the heat tolerance of goldfish (*Carassius auratus*). Can. J. Res., **D27**: 85-91.

HUNTSMAN, A. G. (1945). Migration of salmon parr. J. Fish. Res., Bd. Can., **6**: 399-402.

LUCAS, C. C. and HUTCHINSON, A. H. (1927). A bio-hydrographical investigation of the sea adjacent to the Fraser River mouth. Trans. Roy. Soc. Can., 3rd ser., **21** (sec. V): 485-520.

NASH, J. (1931). The number and size of glomeruli in the kidneys of fishes, with observations on the morphology of the renal tubules of fishes. Amer. J. Anat., **47**: 425-41.

RICKER, W. E. (1938). "Residual" and kokanee salmon in Cultus Lake. J. Fish. Res. Bd. Can., **4** (3): 192-218.

TULLY, J. P. (1942). Surface non-tidal currents in the approaches to Juan de Fuca Strait. J. Fish. Res. Bd. Can., **5** (4): 398-409.

WARD, N. B. (1932). The origin of the landlocked habit in salmon. Proc. Nat. Acad. Sc., **18** (9): 569-80.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLV : SERIES III : JUNE, 1951
SECTION FIVE

A Comparative Study of the Myology of the Head Region in Three Species of Thamnophis (*Reptilia, Ophidia*)

I. McT. COWAN, F.R.S.C., and W. B. M. HICK

FOR well over a century, anatomists have shown a rather sporadic interest in the head region of various members of the Ophidia. Universally, this interest seems to have been aroused in an attempt to describe the bones and muscles constituting the highly complex mechanism responsible for the peculiar feeding activities of some of the species. Among the earliest workers in this field, Duges (1827) gave a description of musculature in connection with a paper on physiology and the swallowing process; Duvernoy (1832) compared the poisonous and non-poisonous forms, with special reference to their comparative head musculature; and d'Alton (1834) published a very detailed account of the musculature of *Python bivittatus.* At the close of the nineteenth and beginning of the twentieth century, Hoffman (1890), Hager (1906), Versluys (1912), and Phisalix (1914), each approaching the field from different aspects, and with different aims in view, made further contributions to the general knowledge of ophidian musculature.

Unfortunately, this work was carried out on a purely descriptive or extremely narrow comparative basis, with little or no attempt at homologizing the muscles in groups with those of the other vertebrate classes. It was not until 1935, when Edgeworth published his *Cranial Muscles of Vertebrates* with a comprehensive synonomy, that some order could be claimed for terminology of vertebrate cranial musculature, and more important here, for ophidian cranial musculature. A previous attempt at finding a basis for homology had been made by Adams in 1915 in his "Memoir on the Phylogeny of the Jaw Muscles in Recent and Fossil Vertebrates." This work, on the jaw muscles only, was undoubtedly valuable as a stimulus to thought on the subject, but due chiefly to his lack of investigation into the embryological aspects of the work, many inconsistencies with the later, more comprehensive work of Edgeworth may be found. The general plan and terminology of Edgeworth has been adhered to in this paper. *Thamnophis* presents a few points of divergence from Edgeworth's plan. Where these occur, special mention has been made and a brief discussion included. Such inconsistencies as to the details of the ground plan of ophidian head musculature are certainly to be expected when one considers the gross inadequacy of the work so far carried out. Edgeworth has combined his own extensive embryological studies, especially of *Tropidonotus natrix*, with the works of the writers already mentioned, and some others, for the basis of the general plan of ophidian head musculature, but all combined can only have dealt with a very few of the large numbers and infinite variety in species among the Ophidia. In fact, the number of species of snakes whose head musculature has been subjected to even a reasonably comprehensive investigation is incredibly small. There seems to have been no work yet published which has adequately described all the muscles usually considered to be within the boundaries of the cranial musculature for any one species. Almost invariably, the adductors of the mandibles, certain portions of the so-called pterygoid musculature, the depressors of the mandibles, and occasionally the intermandibularis have been described; and the remainder of the head seems to have passed into the incinerator along with any ideas the authors may have had about it.

C. P. Gnanamuthu (1937) published a paper on the hyoid and tongue of various reptiles in which he describes the ventral musculature of some nine Lacertilia, two Chelonia, one Crocodilia, and two Ophidia—namely, *Helicops schistcsus* and *Python ṃolarus*—both of the fauna of South India. The paper is, however, dominated by descriptions and comparisons among the lacertilians, and the descriptions of the situation in *Helicops* and *Python* are rather sporadic and in some places almost entirely disregarded. Adams (1925) described the head muscles which he found in *Natrix cyclopion*.

A major work on the head musculature of Sauropsida by Lakjer (1926) dealt with certain head muscles of *Boa constrictor*, *Cylindrophis rufus*, *Typhlops punctatus*, *Tropidonotus natrix*, *Naia trepidians*, *Enhydria hardwickei*, and *Lachesis lanceolatus*, all of the reptilian suborder Ophidia.

It is with this general background, then, that the writers of the present work have entered the study of ophidian cranial musculature. Our aims in working on this subject have been twofold. It is very obvious from what has already been written that there is a broad untouched field for the student of comparative anatomy in the subject of ophidian head musculature. The primary aim in this work has been to describe the cranial muscles of three species of garter snakes, and to attempt to relate the results to the findings of previous workers. In the discussion, evidence which might tend to confirm or contradict the conclusions of other authors with regard to homologies has been suggested, and, where apparent, the special adaptive structure of certain muscles for their probable actions has been considered.

The three species of garter snake chosen for this study inhabit the same region in southwestern British Columbia. Two of them, *Thamnophis elegans vagrans* and *Thamnophia ordinoides ordinoides*, are closely allied and, until very recently were regarded as subspecies within the large, very variable species *ordinoides*. The first named is an active hunter, living largely upon small vertebrates. It is a capable fish catcher and often frequents the beaches, feeding upon the fish in the tide pools. *Ordinoides*, on the other hand, lives almost exclusively on invertebrates of which slugs constitute an important item. *Thamnophis sirtalis tetrataenia*, like *elegans*, feeds in large part on vertebrates. Frogs, salamanders, fish, and small mammals make up the bulk of its diet.

The question arose whether, in these three snakes, the details of cranial musculature would support the apparent systematic affinity between *ordinoides* and *elegans* or whether, perhaps, the similarity in feeding habits and habitat between *elegans* and *sirtalis* would find a reflection in a similar head musculature.

MATERIALS AND METHOD

The description of *T. sirtalis tetrataenia* is based on the dissection of four specimens. Measurements, found below, were taken from but one of these. Where variation occurred between individuals of the subspecies, mention is made in the appropriate places.

One specimen of each of *T. elegans vagrans* and *T. o. ordinoides* was dissected for the comparative study, and such measurements as are mentioned were taken from these.

A skull of *T. s. tetrataenia* was prepared as a basis for understanding muscle relationships, but no detailed work was attempted on it.

The specimen of *tetrataenia* upon which the description is primarily based measured 60 cm. in total length, those of the *elegans* and *ordinoides* were 56 cm. and 50 cm. respectively.

The three garter snakes here considered exhibit some rather striking differences in form of head and some associated structures. These may be briefly described as follows:

Shape of Head

T. s. tetrataenia—head flattened on top; narrowing rounded snout (due to small size of supralabial glands); eyes large; head little wider than rest of body.

T. o. ordinoides—head flattened on top, very much shorter than in *vagrans* or *tetrataenia*; little distinction in width between head and neck; snout rounded due to much enlarged supralabial glands; eyes small.

T. e. vagrans—entire head markedly compressed dorsoventrally; snout pointed; head long and much wider at base than the body region immediately posterior—thus a very obvious neck is apparent.

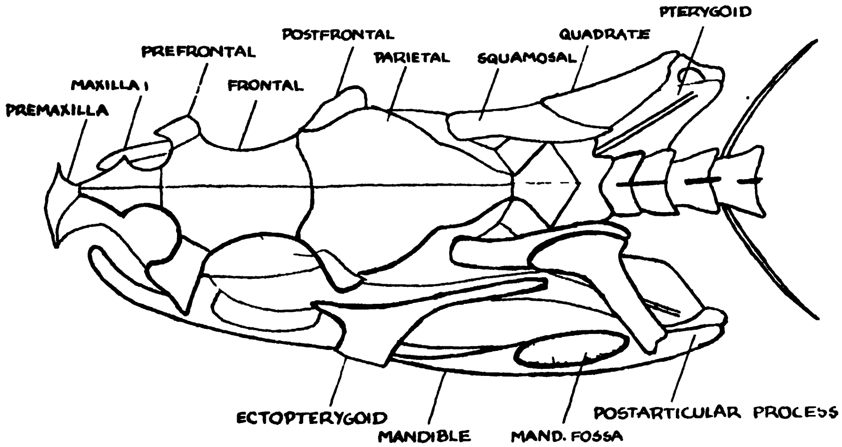


FIGURE 1.—Dorsolateral view of left side of skull.

Jaw Length

This was measured in a straight line from the anterior tip of the dentary to the posterior tip of the post-articular process.

			% of total length
T.s. tetrataenia	—	2.4 cm.	4
T.o. ordinoides	—	1.7 cm.	3.4
T.e. vagrans	—	2.4 cm.	4.3

This contrast in jaw lengths has a considerable effect on the appearance of the muscles attached, especially the adductores mandibulae.

Supra and Infralabial Glands

These are much more highly developed in *ordinoides*, especially at their anterior ends, than they are in *vagrans* or *sirtalis*. This may be correlative to the amounts of lubrication required in swallowing the various types of prey.

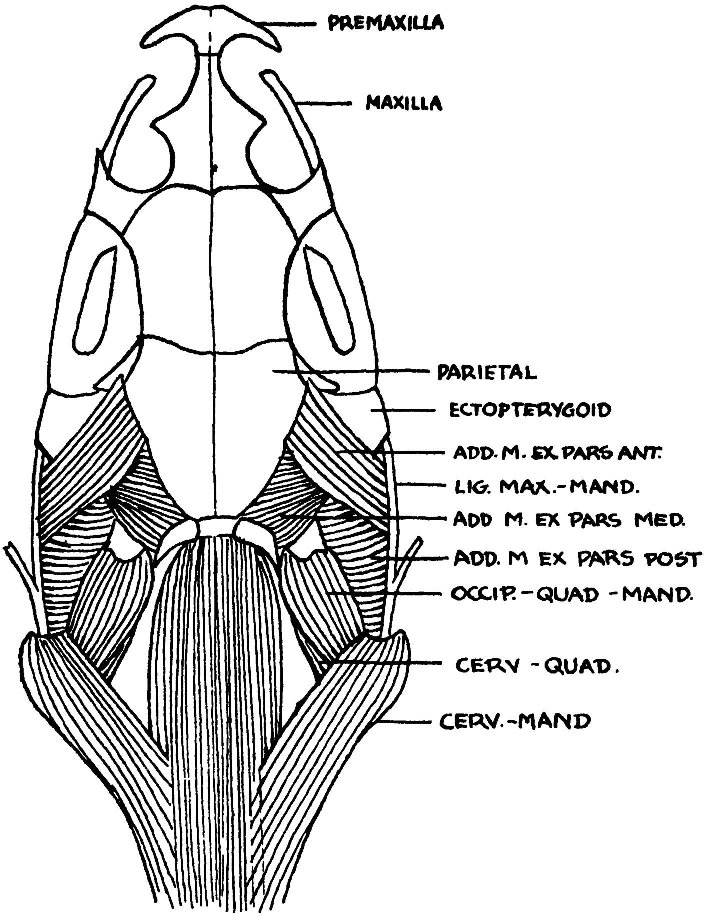


FIGURE 2.– Dorsal view of superficial cranial muscles with supralabial glands and M. constrictor colli removed.

Ligamentum maxillo-mandibulaire (Fig. 2)

This is a shiny white tendinous band arising on the capsule of the jaw-joint, beneath the insertion of M. cervico-mandibularis, and passing directly cephalad. A short distance along its course the band splits into a more lateral strip inserting on the skin, and a medial strip, which continues cephalad, closely apposed to the medial wall of the supralabial gland, to insert on the most posterior projection of the maxillary bone. This ligament shows its greatest development in *ordinoides*.

DESCRIPTION OF THE CRANIAL MUSCLES

The descriptions of the individual muscles are arranged into groups on the basis of common or homologous innervation, on the evidence of comparative myology, and where possible, on developmental data.

THE MANDIBULAR MUSCLES

I. *The Masticatory Muscles*

The movability of the palatoquadrate in the Sauropsida is accompanied by a division of the masticatory muscle plate into a constrictor dorsalis and an adductor mandibulae. The former passes from the chondrocranium to the movable palatoquadrate, the latter from the chondrocranium to Meckel's cartilage. There is a marked parallelism between the degrees of movability of the palatoquadrate and the development of the pterygoid musculature, i.e., the end products of the constrictor dorsalis. Thus it is obvious that in such a group as the Ophidia, where kinetism and streptostylism have reached such a marked degree of specialization, the muscles involved will be correspondingly divided and specialized, with changed origins and insertions to suit the needs of the complex operations involved. Thus we find much greater complexity in the end products of the primordial mandibular sheet than, for instance, in crocodiles or turtles, with akinetic skulls, or in lizards, where kinetism has not proceeded to the same extent as in snakes.

1. THE ADDUCTORES MANDIBULAE. In genus *Thamnophis*, as in most Ophidia, this mass has become divided into three main groups, each of which shows subdivision.

(i) M. adductor mandibulae externus (Figs. 2, 6). This constitutes the largest muscle mass of the head and is subdivisible into three parts. All the fibres pass from the skull or quadrate, ventrad to insert on the mandible.

(*a*) pars anterior. This is the smallest of the three divisions of the add. mand. ex.

Origin—in a groove formed by a lateral ridge of the parietal and the dorsal posterior portion of the postfrontal.

Insertion—on the lateral surface of the mandible. This muscle is a narrow band, thicker at its origin than at its insertion. The fibres pass obliquely ventrad and caudad, and, about one-half way along their course, change direction to pass directly ventrad to their insertion. This change of fibre direction is supported by an aponeurosis extending on each side to blend with the fascia over the underlying add. mand. ex. pars posterior.

This muscle lies superficial to all others in the area, overlying the add. mand. ex. pars posterior at its insertion, the add. mand. ex. pars medius along its course, and a large post-orbital gland at its origin.

In *elegans*, the origin of this muscle is extended further ventrally on the post-frontal and further posteriorly on the parietal than it is in *sirtalis*. In line with the general flattening of the head in this subspecies, the fibres are less oblique in their posteriorly directed course, thus overlying more of the add. mand. ex. pars medius.

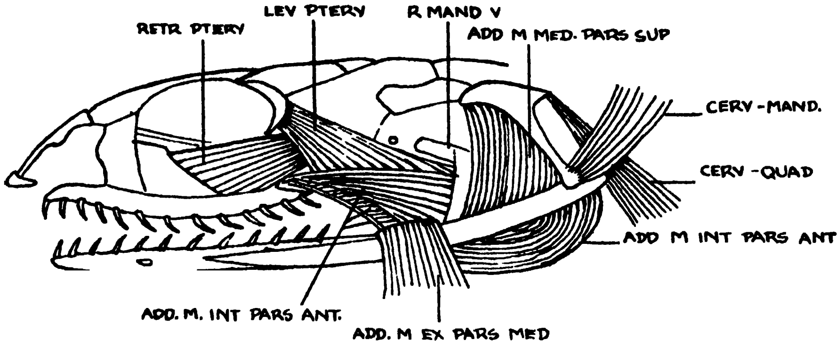


FIGURE 3.—Lateral view of deeper muscles of left side.

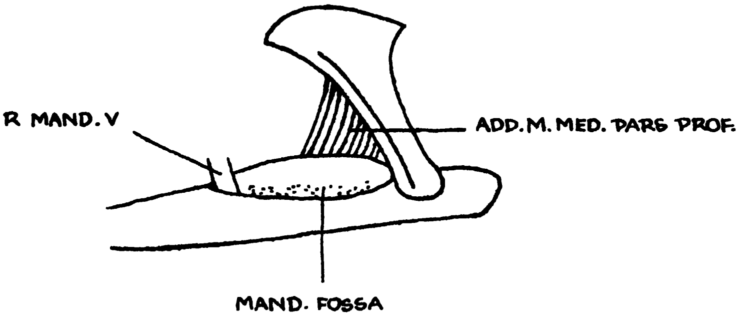


FIGURE 4.—Quadrate-articular joint of left side. Lateral view to show M. add. m. med. pars prof.

(*b*) pars medius.

Origin—on the dorsolateral surface of the skull in a deep, well-defined groove provided by the parietal and supraoccipital.

Insertion—in a broad sheet on the dorsal surface of the mandible from the anterior tip of the mandibular fossa to the anterior limit of the add. mand. mass insertion, i.e., the beginning of dentition on the mandible.

The mandibular root of the fifth cranial nerve passes down just posterior to, and in line with, the posterior fibres of this muscle, entering the mandibular fossa at its anterior limit.

(*c*) pars posterior. The largest of the three divisions of the add. mand. ex. It is a thick triangular sheet of muscle fibres (assuming the quadrate-articular joint is the apex of the triangle).

Origin—the entire length of the anterior surface of the quadrate. The chief origin of fibres is on the anterior projection of the proximal articulation of the quadrate and, in common with the posterior fibres of the add. mand. ex. pars medius and certain fibres of the occ. quad. mand., on the tough ligament which overlies the quadrate-squamosal articulation and passes up to the supraoccipital crest.

Insertion—entirely on the lateral surface of approximately the posterior half of the mandible.

Comparing the three subspecies, *ordinoides* has the most compact and heaviest add. mand. ex., but the antero-posterior length of the insertion is about one-third the total jaw length, whereas in *elegans* and *sirtalis* the insertion of the mass covers about one-half the total jaw length. When the jaws are closed, there is a conspicuous bulge in the add. mand. ex. pars posterior of *ordinoides* lacking in the other two forms. In correlation with the shorter and stouter quadrate in *ordinoides*, with its very broad proximal articulation, the add. mand. ex. pars posterior has an even stronger origin, especially at its dorsal anterior angle. The length of the base of the triangular add. mand. ex. pars posterior is least in *elegans* and greatest in *ordinoides*.

In the main, details of origin and insertion coincide in the three forms, and any modification in shape is correlative with a corresponding modification in skull shape.

(ii) M. adductor mandibulae medius (Figs. 3, 4). This muscle group constitutes a triangular sheet of fibres lying deep to the add. mand. ex. pars posterior, all very similar in shape, fibre direction, origin, insertion, and thickness except that the most anterior fibres, or base of the triangle, do not extend as far anteriorly as is the case in add. mand. ex. pars posterior. The muscle is subdivisible into two distinctly separate sheets, both with origin on the quadrate and insertion on the mandible.

(*a*) pars superficialis. The larger of the two sheets, bounded anteriorly by the mandibular root of the fifth cranial nerve, which passes ventrad into the anterior end of the mandibular fossa, wedged between the add. mand. ex. pars medius and this muscle along the ventrally directed portion of its course.

Insertion—fills the entire mandibular fossa, with the exception of the anterior tip which is occupied by the mandibular root of the fifth cranial nerve in its passage into the Meckelian fossa.

(*b*) pars profundus. A small, thin, triangular sheet of fibres whose anterior boundary lies a few mm. posterior to the anterior limits of the pars superficialis. The fibre direction is the same, i.e., vertical, the fibres becoming progressively shorter from anterior to posterior limits (as is, of course, the case in pars superficialis and add. mand. ex. pars posterior).

Insertion—solely on the medial dorsal surface of the mandible, i.e., along the upper medial edge of the coronoid.

This muscle group shows very little diversity in any of the specimens examined, except that in *elegans* it seems to be comparatively weaker in respect to the rest of the add. mand. mass.

(iii) M. adductor mandibulae internus. This group constitutes the deepest, and undoubtedly the most specialized of the muscles of the add. mand. sheet.

(*a*) pars anterior (Fig. 5). A large pyriform muscle lying wedged in between the mandible and the pterygoid.

Origin—on the postero-lateral edge of the shield-shaped distal end of the ectopterygoid. The more lateral fibres are collected into a bundle and partially surrounded by a tendinous sheath, while the more medial fibres arise in a thin, flat sheet on the dorsal surface of the postero-lateral edge of the ectopterygoid.

Insertion—the more medial sheet of fibres passes obliquely postero-lateral to insert in a line along the medial ventral surface of the posterior third of the mandible.

The fibres arising in the bundle insert on the posterior end of the mandible on its ventro-mesial surface.

(*b*) pars posterior (Fig. 5). This smaller and far less conspicuous part of the two subdivisions of this group is a flat sheet of fibres passing from the pterygoids almost directly laterally to the posterior tip of the mandible.

Origin—along the dorsolateral groove of the pterygoid and that portion of the ectopterygoid which overlies the pterygoid (approximately its proximal third) also on the posterior tip and lateral edge of the ventral surface of the pterygoid.

Insertion—in the postero-medial fossa of the articular.

No noteworthy variations in the structure of this muscle group were observed in either of the other two forms.

2. THE CONSTRICTORES DORSALIS. In genus *Thamnophis*, as in most Ophidia, this inner mass of the primordial mandibular muscle plate has become divided into five individual muscles.

(i) M. retractor vomeris (Fig. 5). A very inconspicuous pair of

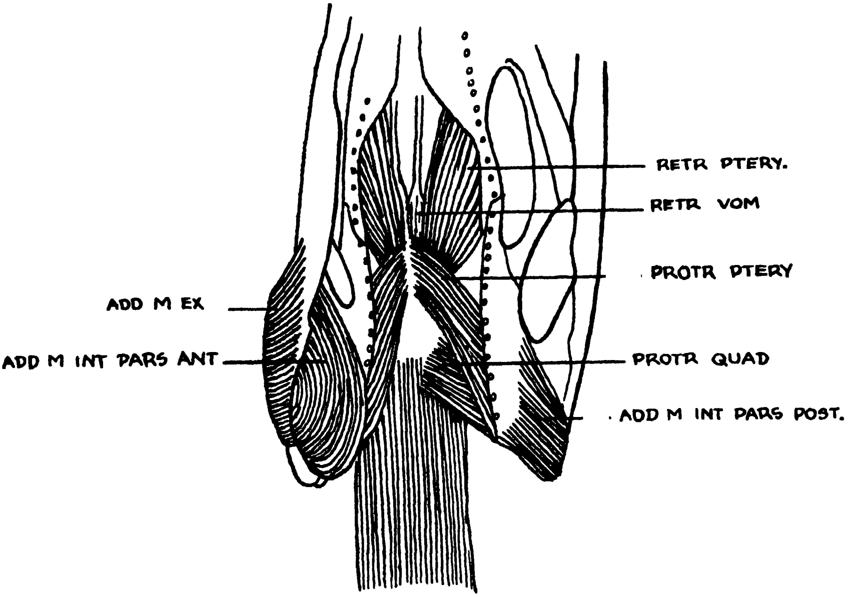


FIGURE 5.—Ventral musculature of the skull. Intermandibular and hypobranchial spinal musculature together with entire buccal membrane removed Left mandible displaced laterally

muscles lying closely approximated to each other in the mid-ventral line of the anterior half of the skull. There is a short broad base on the anterior side of the ventral ridge of the basisphenoid between the origins of the retractor pterygoidei of each side. The muscle narrows down rather abruptly, a short way along its course, to a tendon, which passes directly anterad, inserting on the posterior tip of the vomer.

(ii) M. retractor pterygoidei (Figs. 3, 5). A short, stout band of fibres appearing, in lateral view, to fill the postero-ventro-mesial corner of the orbit.

Origin—from the anterior side of the ventral ridge on the basisphenoid, lateral to the origin of the retractor vomeris. This origin is quite broad, extending dorsolaterally to the limit of the ridge.

Insertion—fibres pass antero-lateral in a thick band to insert on the entire, free, medial edge of the palatine.

(iii) M. levator pterygoidei (Fig. 3). The largest and most superficial muscle of this group, appearing, in lateral aspect, as a large fan-shaped sheet.

Origin—in a notch provided by the post-frontal and pleurosphenoid at the most ventral part of their line of fusion.

Insertion—the fibres, which arise in a compact bundle, spread out fanwise into a broad, flat sheet which inserts along the entire length of the dorsomedial fossa of the pterygoid.

(iv) M. protractor pterygoidei (Fig. 5). A stout band of muscle fibres about equal in size and similar in structure to the retractor pterygoidei.

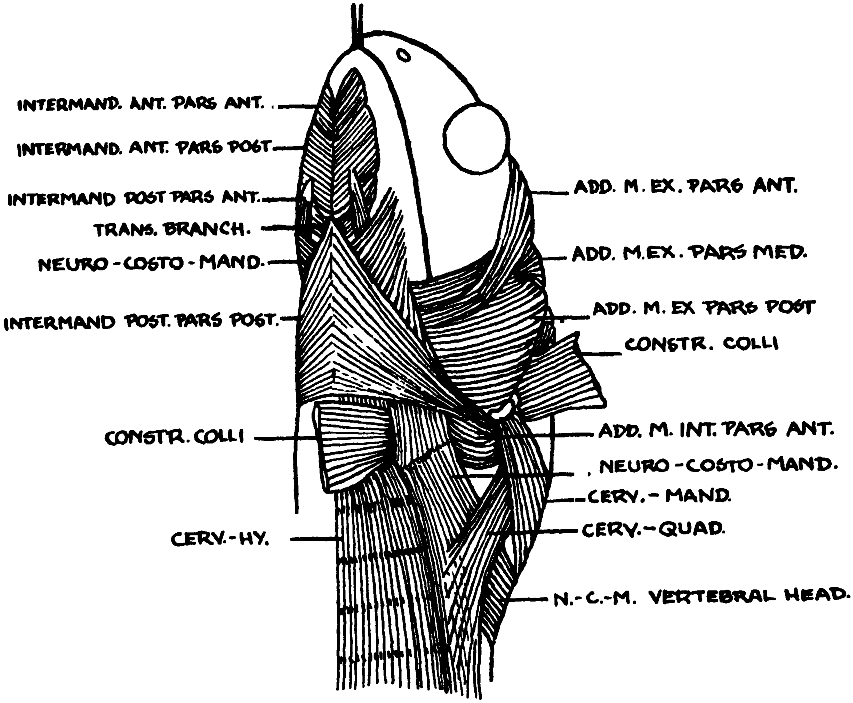


FIGURE 6.—Ventro-lateral view of the superficial muscles of the left side. M. constrictor colli cut and laid back.

Origin—on the posterior side of the basisphenoid ridge and on the mid-ventral line of the basisphenoid a short distance posterior to this.

Insertion—fibres pass postero-laterad, following the course of the pterygoid bone. A few lateral fibres insert on the tough membrane surrounding the pterygoid teeth and these hold the muscle closely to the pterygoid. The great majority of these fibres pass postero-laterad to insert on the posterior tip of the pterygoid and on the tough ligament which joins the tip of the pterygoid to the post-articular process.

(v) M. protractor quadrati (Fig. 5). A small, thin sheet of muscle fibres lying deep to all other muscles in the area. This is the most posterior of the cranial muscles to take origin from the rigid portion of the skull.

Origin—from the posterior end of the basioccipital and the fascia over the ventral semispinalis muscles.

Insertion—fibres pass in a thin, almost parallel band to insert on the post-articular process of the mandible. Fibres of the protractor pterygoidei cover this muscle completely from every aspect if the skull is left in its normal positition of rest.

II. *The Intermandibular Muscles*

The lack of a symphysis at the tip of the mandibles in Ophidia, together with their extreme separation in swallowing food, makes the intermandibularis musculature of the snakes very important. The relations of these muscles concerned in bringing together (or adducting in a different sense) the mandibles after separation, are not very clearly understood in their normal position, but when the mandibles are spread apart, their functional adaptations are clear.

In the specimens examined, this group has become subdivided into three sub-groups and these into five individual muscles.

(i) M. intermandibularis anterior (Fig. 6, 7, 8). Although the subdivision in this muscle group is complete, the two individual muscles formed appear superficially to be continuous. Both lie superficially at the anterior tip of the mandibles, with considerable attachment of their fasciae to the skin over that area.

(*a*) pars anterior. A thick, triangular-shaped muscle lying ventral to the swellings on either side of the glottis.

Origin—ventral surface of the anterior tip of the mandible.

Insertion—into a mid-ventral intermandibular ligament and on the buccal membrane and/or the cartilaginous ring surrounding the glottis (see Fig. 8).

(*b*) pars posterior. A short flat band of muscle, very similar in structure and course to the pars anterior, and lying very closely apposed to it anteriorly.

Origin—ventro-mesial surface of the mandible immediately posterior to the origin of pars anterior. The origin lies directly over (from the ventral aspect) the origin of the lateral head of the genioglossus and the geniotrachealis.

Insertion—fibres from each side pass obliquely postero-mediad to insert in a mid-ventral raphe and on the fascia over the tongue sheath with its median gland. The aponeuroses which form this raphe are continuous posteriorly with those of the transversus branchialis.

(ii) M. transversus branchialis (Fig. 7). A very thin, broad, flat sheet of fibres arising in an antero-posterior line on the buccal membrane of the floor of the mouth. The fibres pass ventrad, and then postero-mediad around the juncture of the two heads of the genioglossus to insert in a median raphe over the mid-ventral line just posterior to and continuous with the mid-ventral raphe of the intermand. ant. pars posterior.

(iii) M. intermandibularis posterior (Fig. 6, 7). The two individual muscles which have become divided to form this group bear far less similarity to each other than the two subdivisions of the intermandibularis anterior. However, their general relationships indicate a common origin and they will be considered in this light here.

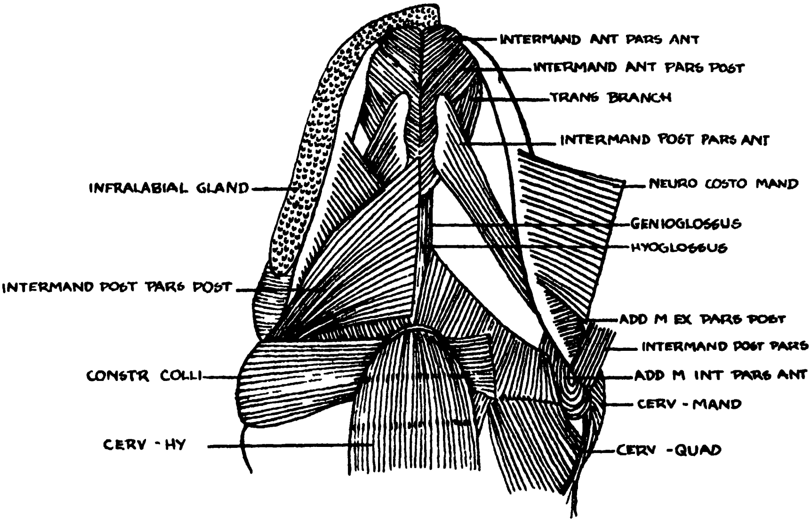


FIGURE 7.— Ventral view of head, especially to show the intermandibular musculature. M. intermand. post. pars posterior of left side removed and M. neuro-costo-mand. of left side cut and laid back. Both mandibles displaced laterally.

(*a*) pars anterior. A long, thin, flat band of fibres of equal width throughout. This muscle lies deep to the insertion of the neuro-costomandibularis, and is very closely apposed to the medial face of the mandible when at rest, but, if the mandibles are spread, the relationships of this muscle become apparent.

Origin—the fibres arise directly from the medial surface of the mandible about half-way along its length.

Insertion—fibres pass antero-mediad in a flat band to insert by a broad aponeurosis over the ventral fascia of the transversus branchialis and intermand. ant. pars posterior.

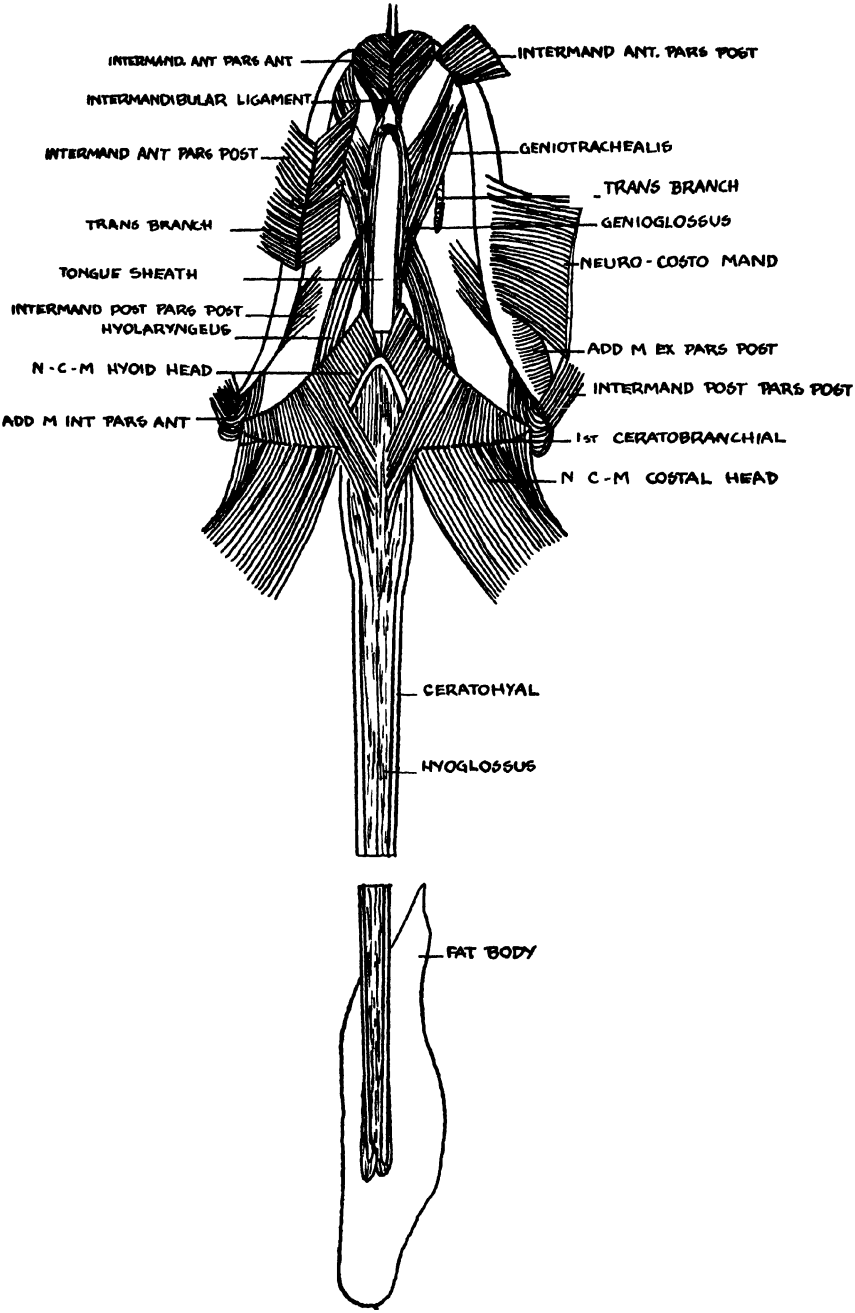


FIGURE 8.—Deeper portions of the ventral musculature especially to show the relationships of the hyoid and lingual apparati Both mandibles displaced laterally.

(*b*) pars posterior. A thin triangular sheet of fibres having a narrow origin by digitations from the ventro-mesial surface of the post-articular process. The fibres spread out fanwise from this origin in a mediad, or in the case of the more anterior fibres obliquely antero-mediad, course to insert in a mid-ventral raphe. The aponeuroses of this raphe are continuous with those of the trans. branch. and inter-mand. ant. pars posterior. The anterior tip of the insertion overlies that of the trans. branch., and by its rapheal continuity lies closely apposed to it. The origin is very closely apposed to the insertion of the add. mand. int. pars anterior on the mandible. The insertion is limited posteriorly by the basihyal.

The only noteworthy variations within the three species occurred in the intermand. post. pars posterior. In *elegans*, this muscle is even better defined than in *sirtalis*, whereas in *ordinoides* the muscle appears very thin and weak. In *elegans* the muscle is bound laterally to the mandible over its entire length by a fascia and a few of the more anterior fibres arise from this anterior extension of the muscle's attachment to the mandible. However, the greatest origin of fibres is as described in *sirtalis*. In both *elegans* and *ordinoides* the posterior fibres of this muscle were found to be continuous with the anterior fibres of the constrictor colli, but in *sirtalis* the anterior fibres of the constrictor colli overlie the entire origin of this muscle.

THE HYOID MUSCLES

From a comparative embryological study of this musculature in various members of the vertebrate classes, Edgeworth has concluded that in the primitive sauropsidan stock the hyoid muscle plate, which developed as a narrow vertical band of cells, extended backwards into the opercular fold forming a broad sheet, the constrictor colli, and that fibres separated from the dorsal part of its anterior edge formed a levator hyoidei and a depressor mandibulae.

It may be assumed that here, as in most other vertebrates, the innervation of this group is via the seventh cranial nerve. However, if our assumptions regarding the hyoid head of the neuro-costo-mandibularis muscle are correct (see below), an additional innervation from the spinal nerves would follow for this muscle.

I. *Constrictor Cólli*

(i) M. constrictor colli (Figs. 6, 7). This is represented by a broad, thin, sheet of vertical fibres passing around the "neck" superficial to all other muscles in this area.

Origin—from two heads on the dorsal surface. The dorso-anterior portion arises from the outer surface of the quadrate, the fibres passing out between the add. mand. ex. pars posterior and the occipito-quadrato-mandibularis. The fibres from this head join immediately with those of the main sheet, which arises from the fascia over the dorsal spinal muscles, and the occ.-quad.-mand.

Insertion—the entire muscle passes ventrad, following the curve of the "neck," to insert under the cervico-hyoideus portion of the rectus cervicis musculature on the anterior ends of the ceratohyals, just posterior to their fusion. This insertion lies ventral to the origin of the hyoid head of the neuro-costo-mand.

This muscle is represented best in *sirtalis* for in *elegans* and *ordinoides* no evidence of its dorsal half was found.

In *elegans* the muscle arises on the postero-lateral surface of the tip of the articular and from the fascia over the neuro-costo-mand. In *ordinoides*, the origin seems to be solely on the lateral surface of the distal end of the quadrate and on the capsule of the quadrate-articular joint. In both, the anterior fibres of this muscle are continuous with the posterior fibres of intermand. post. pars posterior, and the insertion in both is similar to that described for *sirtalis*.

II. *The Depressores Mandibulae*

This group has become divided into three separate muscles, two of which are comparatively simple, and the other very complex.

(i) M. occipito-quadrato-mandibularis (Fig. 2). This stout sheet of fibres covers the entire outer surface of the quadrate, overlapping its posterior edge and covering a small portion of the inner surface of the quadrate.

Origin—on the lateral surface of the proximal end of the quadrate, its posterior projection, and on the tough ligament overlying the quadrate-squamosal articulation.

Insertion—fibres follow the direction of the quadrate, lying in the lateral fossa provided by it, and pass postero-ventrad to insert in a bundle in the deep notch provided on the dorsal surface of the post-articular process.

In *elegans*, as in *sirtalis*, the muscle arises entirely from the quadrate. The greater part of the muscle does not, however, lie on the quadrate. The more posterior portion of the muscle contains by far the most fibres, this concommitant with an increase in size of the projections on the proximal end of the quadrate in both *elegans* and *ordinoides*.

In *ordinoides*, the majority of fibres arise on the exoccipital, a few more anterior fibres arising on the squamosal, and the only origin on the quadrate is that of the fibres arising from its inner surface.

(ii) M. cervico-mandibularis (Figs. 2, 3, 6, 9). A broad, flat sheet of muscle arising from the fascia over the dorsal spinal muscle from the third to fifth cervical vertebrae. The fibres pass antero-ventrad converging to a flat tendinous insertion on the capsule of the jaw-joint.

(iii) M. neuro-costo-mandibularis (Figs. 6, 8, 9). A very large sheet of muscle fibres extending over a larger area than any other single muscle in the head region.

Origin—from three main heads:

Vertebral head—from the fascia over the dorsal spinal muscles from the sixth to the eighth vertebrae (thus lying immediately posterior to the origin of m. cervico-mand.).

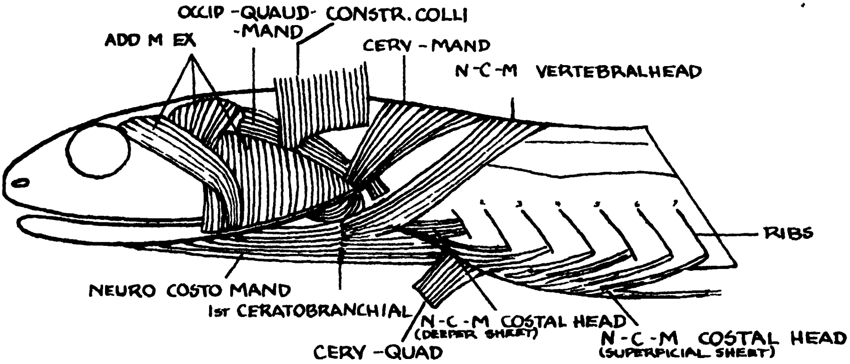


FIGURE 9.—Lateral view of head with costal head of M. neuro-costo-mand. dissected out to show its origin from the ribs. M. intermand. post. pars posterior and M. cervico-hyoideus have been removed and M. constrictor colli and M. cervico-quadratus cut.

Costal head—by slips from approximately the first six anterior ribs (variation with different specimens). These slips unite into two sheets: a superficial sheet whose fibres arise medially on the most posterior of the ribs used for the origin and also on the fasciae of underlying muscles; a deeper sheet, the more medial part of which arises on ribs posterior to those from which the more lateral fibres arise.

There is considerable variation as to the ultimate origin of these slips, some ribs having very few fibres arising on them, while the next may bear the origin of two slips. However, the general pattern is the same, and the number of slips varies little from six.

The superficial sheet of this head enters the main body of the muscle in a transverse line along the first ceratobranchial, appearing to be inserted into this cartilage, but actually the fibres may be shown to continue on with the main body of the muscle to its common insertion.

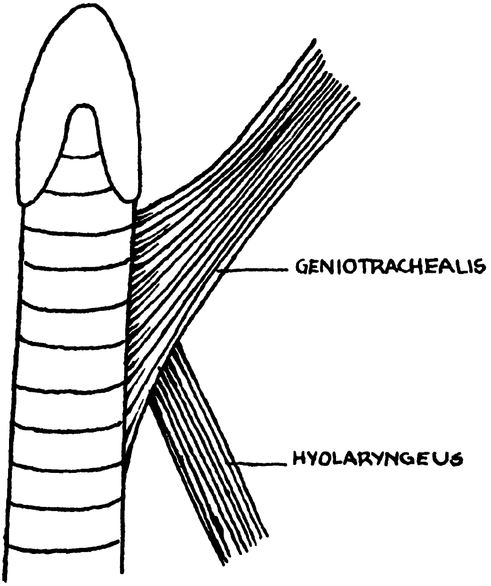


FIGURE 10.—Ventral view of trachea and larynx to show relationships of its extrinsic musculature.

Hyoid head—this most medial head arises from the anterior ends of the ceratohyals dorsal to the insertion of the constrictor colli, and from the basihyal in a flat sheet of fibres which immediately becomes continuous with the main body of the muscle passing antero-laterally at this point. The fibres of this head form the most medial portion of the main muscle body. Certain fibres form the fascia of the hyoglossus, passing over the ceratohyals to become absorbed by the body of the hyoid head.

The vertebral head passes antero-ventrad to join the costal head (deeper or main portion) at approximately the region of the second ceratobranchial and the hyoid head enters this combination just anterior to the first ceratobranchial (or the fusion of the two sheets of the costal head).

Insertion—after all three heads have united, the muscle passes antero-laterad, deep to the intermand. post. pars posterior but overlying all other muscles in the area. The insertion is by a broad tough aponeurosis on the ventral surface of the mandible. This insertion extends anteriorly from approximately the anterior limit of the add. mand. insertion and lies under the ventral edge of the infralabial gland. It extends anteriorly to approximately the middle of the dentary-angular joint.

In *ordinoides* and *elegans*, only minor differences were noted in the heads of origin. The costal head (pars superficialis) seemed more pronounced in *ordinoides* but the situation in both parallels that of *sirtalis* very closely. The insertion of the muscle in *elegans* which corresponds to that described for *sirtalis* is very weak, the main insertion being on the medial surface of the mandible by a tough aponeurosis and a few fibres also inserting on the buccal membrane in this region. The antero-posterior limits of the insertion remains, however, unchanged. The insertion in *ordinoides* shows little variation from that found in *sirtalis.*

THE HYPOBRANCHIAL SPINAL MUSCLES

In the Ophidia, the hypobranchial musculature falls into an anterior geniohyoideus group and a posterior rectus cervicis group. However in the absence of the pectoral girdle the latter has become more or less continuous with the ventral musculature.

I. *Rectus Cervicis Group*

This posterior group has become divided into two muscles in these forms, both arising on the fasciae of the ventral musculature.

(i) M. cervico-hyoideus (Figs. 6, 7). A broad band of fibres extending posteriorly from the basihyal.

Origin—on the fascia over the costal head of neuro-costo-mand., the general fasciae in the "neck" region, and the anterior edges of the gastrosteges.

Insertion— on the posterior edge of the basihyal and medial edge and ventral surface of the anterior end of the ceratohyal.

The muscles of each side are continuous medially, and the whole constitutes a superficial sheet of fibres closely adhering to the skin. Laterally, the fibres overlie a considerable portion of the costal head of the neuro-costo-mand.

(ii) M. cervico-quadratus (Figs. 3, 6, 9). A small, thin band of muscle having a broad origin on the skin and fascia over the costal head of the neuro-costo-mand. The fibres converge as they pass dorso-anterad to a tendon which inserts on the posterior projection of the proximal articulation of the quadrate.

No major differences were noted for either muscle in the forms examined.,

II. *Geniohyoid Group*

This muscle group has four individual members in genus *Thamnophis* as follows:

(i) M. genioglossus (Fig. 8). A very long, thin band of muscle.

Origin—from two main heads:

Lateral head—a thin band of fibres arising by an aponeurosis from the mandible directly dorsal to the origin of the much stouter geniotrachealis.

Medial head—from the postero-lateral projections of the intermandibular ligament in a small bundle. A few subsidiary fibres appear to arise from the buccal membrane in this region and others cross over the tongue sheath (forming a "bridge" across the ventral surface of the tongue sheath) to enter the muscle of the opposite side. Both these variations were observed in only one specimen of *sirtalis*, and are probably not consistent throughout the species.

The medial head passes directly posterad alongside the tongue and is very soon joined by the postero-medially directed fibres of the lateral head, the whole muscle then passing backward as a narrow band closely apposed to the tongue sheath and ventro-lateral in relation to it. As it proceeds posterad, it comes to lie lateral to the hyoglossi. The muscles on each side become more and more attached to the fascia of the hyoglossi until, about one-third of the way back, the hyoglossi and the genioglossi on each side become enveloped in the fascia of the hyoglossi; this general point may be considered as their insertion.

(ii) M. hyoglossus (Fig. 8). The hyoglossi of each side are united throughout almost their entire length to form a cylindrical organ, surrounded in its anterior fourth by a reduplicature of the buccal membrane forming a buccal sheath. The two muscles arise separately on the posterior tips of the ceratohyals, but soon after fuse to form a single element which passes anteriorly in the mid-line dorsal to the cervico-hyoideus and ventral to the trachea, passing into the tongue sheath just anterior to the basihyal (which lies ventrally, on its fascia). The anterior tip of the muscle becomes bifid and passes out into the oral cavity via an opening in the anterior end of the buccal sheath, so that the entire anterior one-third of the muscle is capable of protrusion on contraction of the genioglossi.

The origin lies over a large fat body and is connected to it by fascia.

The average length of this muscle in the specimens examined approximated 10 per cent of the total length of the animal.

(iii) M. geniotrachealis (Figs. 8, 10). A large stout band of muscle fibres approximately parallel in its course to the intermand. ant. pars posterior and the lateral head of the genioglossus. It is the deepest of this group of three muscles which successively overlie each other.

Origin—medial surface of the anterior end of the mandible. This is the most dorsal of the three muscles arising in the hollow provided on the mandible in this area.

Insertion—the fibres pass postero-mediad and slightly dorsad to insert over a broad area, in an antero-posterior line, on the lateral surface of the trachea, just posterior to the cartilages of the larynx and postero-ventral to the insertion of the hyolaryngeus.

(iv) M. hyolaryngeus (Figs. 8, 10). A narrow band of muscle, varying considerably in size among the specimens examined.

Origin—by two heads from the hyobranchial skeleton: about the middle of the first ceratobranchial dorsal to the neuro-costo mandibularis: from the ceratohyal laterally or its point of junction with the first ceratobranchial, dorsal to all other muscles in this vicinity.

These two heads unite immediately to form a band of muscle of varying thickness which lies closely adherent to the buccal membrane dorsally in its antero-mediad course.

Insertion- the band of fibres becomes free from the buccal membrane where it passes dorsal to the geniotrachealis and inserts just anterior and dorsal to it, on the lateral wall of the trachea at about the posterior limit of the cricoid cartilage.

Where this muscle crosses the geniotrachealis, dorsally, there may be a considerable intermixing of the fibres, especially at their lateral borders. This condition was observed in only one specimen of *sirtalis* and may be considered unusual.

Discussion

Consideration of Description and Homologies of the Cranial Muscles of *T. sirtalis tetrataenia*

Before proceeding to a discussion of the peculiarities of each individual muscle, a brief consideration of the possibilities of movement in the skull of *sirtalis* seems pertinent.

It should be recognized from the first that the original concept of the word "streptostylism" (first employed by Stannius in 1856 in reference to the fact that in some vertebrates the quadrate bone is movable relatively to neighbouring bones, particularly the squamosal) must be broadened considerably if we are to consider, as some authors have, the entire movement of bones in the ophidian skull as under the single head of "Streptostylism." To avoid putting this extra meaning into the original concept, then, the later term of "kinetism" (De Beer, 1937), referring to the movement of the "maxillary segment" on the "occipital segment," will be adopted here.

In reference to the possibilities for movement in the kinetic skull, De Beer (1937, p. 426) has divided it into two regions, moved on each other by the protractor and levator pterygoid muscles. These consist of the brain case or occipital region, involving prootic, opisthotic, basisphenoid, parasphenoid, basi-, ex-, and supra-occiptals, and the *maxillary region, involving* the quadrate, quadratojugal, jugal, *squamosal, maxilla, premaxilla,* ectopterygoid, pterygoid, palatine, vomer, nasal, frontal, and parietal. In *sirtalis*, then, the occipital region should be considered as the only rigid part of the skull, but further examination indicates that the frontals, nasals, and parietals should also be included in the occipital region, as there seems very little possibility for movement at their junctions. In Ophidia, no quadratojugal or jugal appear, and as the frontals, nasals, and parietals have been consigned to the rigid region of the skull, the quadrate, squamosal, maxilla, premaxilla, ectopterygoid, pterygoid, palatine, and vomer—to which will be added the mandibles as a whole—remain to constitute the more or less movable maxillary region. This maxillary region may be subdivided into three main segments of movement:

(i) *Premaxillary Segment.* The premaxillae and vomers represent a segment with some degree of movement—moved by the retractor vomeris muscles which also aid the segment in holding.

(ii) *Maxillary Segment.* The maxilla, palatines, pterygoids, and ectopterygoids (the first three mentioned bearing teeth) represent a highly mobile group. The maxillae are attached by ligaments to the premaxillae, prefrontals, palatines, and ectopterygoids. The articulations in this segment are mostly of the flat contact type, especially those of the ectopterygoid, an arrangement that allows much opportunity for motion. The maxillae are partly dependent, in action, on the musculature of the palatine and prefrontal; their protracting, retracting, and lateral motions arise largely from this source. Since both the palatine and pterygoid are toothed, they perform the function of holding the prey. The palatines and pterygoids move together, transmitting their movement to the maxillae by the articulation of this bone to the pterygoid via the ectopterygoid. The pterygoid derives movement from its muscles and attachments as well as by a very tough ligamentary connection to the mandible at its posterior tip.

(iii) *Mandibular Segment.* Made up of the squamosal, quadrate, and entire mandible, this segment is capable of a wide range of movement from its many and varied muscle attachments. The articulation of the squamosal to the skull seems fairly rigid, but that of the quadrate to the squamosal is of the flat contact type and that of the quadrate to the articular of the hinge-joint type, both of these latter being capable of a considerable range of movement though restricted mostly to one plane. From the connection between the pterygoid and the mandible, it may be seen that this segment and the maxillary segment may act on occasion together—in fact any movement of either segment will affect the other in so far as its muscle attachments co-operate.

Since these specializations in the articulations of many of the skull bones are so clearly related to the feeding habits of the animal, some explanation of the process involved in food getting may be needed to explain their peculiar uses. Briefly, the whole mechanism required for swallowing centres around the fact that the food object being swallowed may be larger than the snake's head at rest. To accomplish this, the snake makes use of protracting, retracting, and lateral movements of each half of the mandibular and maxillary segments to "crawl" over the prey. The lack of a mandibular symphysis, and the ability of the maxillary and mandibular segments to spread laterally, enable the snake to engage and swallow an object of greater diameter than its own resting buccal cavity. Owing to the elasticity of the distended buccal cavity, a considerable pressure will be brought to bear on the struggling prey being swallowed, which undoubtedly assists in stifling and subduing it. Thus this elaborate provision of extra articulations between many of the skull bones, and the corresponding movements which are made possible, give the ophidian a great advantage in food capture and swallowing.

With this consideration of the possibilities for movement in the bones of the skull, it will be obvious that the musculature of the head must be very complex and extraordinary if the maximum use is to be made of these elaborate articulations. It should be emphasized that many of the muscles described in this paper are neomorphic in the Ophidia, having arisen as divisions from the less complex muscle masses of the ancestral forms. They represent the end-point in this type of specialization and, as such, they cannot be directly homologized to individual muscles of either lower or higher groups, although the parent mass may be very readily homologized to that of other groups.

THE MANDIBULAR MUSCLES

I. *The Masticatory Muscles*

1. The Adductores Mandibulae. The external and medial portion of this group is referred to by Adams (1915, 1925) as the capitimandibularis mass, and the internal as the pterygoideus anterior and posterior. (Almost every worker has applied a new name to the individual muscles in this and the other groups, and the full synonymy, which may be found in Edgeworth (1935), will not be repeated here.) There may be some justification for Adams's separation of the add.

mand. ex. and medius from the internus if functions, origins and insertions, and general fibre direction only are considered. However, Edgeworth has demonstrated that in the embryonic development of this muscle mass in vertebrates with a movable palatoquadrate, a division takes place which splits the mass into an add. mand. and a constrictor dorsalis. Adams states that his capiti-mandibularis mass is homologous throughout the vertebrates, but Edgeworth (1935) has shown that in Dipnoi, Holocephali, Amphibians and Mammals, all of which retain the fixed palatoquadrate, no transformation of the dorsal region of the masticatory muscle plate takes place, and consequently no one muscle of this group in the adult can be homologous with any single muscle in the Sauropsida, a statement which contradicts that made by Adams.

Thus little agreement as to the homologies of the individual muscles can be expected when even the homologies of the parent masses are not agreed upon.

(i) M. adductor mandibulae externus

(*a*) pars anterior. This division seems to have been most generally referred to as the masseter, but, as has been shown above, such a homology to this typically mammalian muscle is quite impossible if Edgeworth's findings are accepted.

(*b*) pars medius. Most frequently referred to as the anterior portion of a temporalis.

(*c*) pars posterior. Constituting the posterior portion of this temporalis according to some writers. Again this homology is based on rather imaginative superficial resemblance, and has no developmental support. The best mechanical efficiency is obtained in this muscle by having its strongest origin near its anterior limits.

All three parts of this group act primarily to adduct the mandible.

The partes medius and posterior are by far the strongest, and the pars anterior, by reason of its weak insertion, may be considered of little functional value. This may indicate that the muscle is passing out of existence in these forms. The fact of its insertion lateral to pars medius and posterior, instead of anterior to them as we might expect, may be due to a posterior extension of the dentition of the mandible.

(ii) M. adductor mandibulae medius. Two divisions of this muscle are described, a condition not recorded elsewhere. To these distinctly separate muscles the names add. mand. medius pars superficialis and pars profundus have been applied because of their lateral relationships to each other. The question will undoubtedly arise as to why it has been assumed that the add. mand. medius, and not the add. mand. ex. pars posterior, has divided. The origins and insertions, or fibre direction, certainly give little clue unless one supposes that because both the assumed derivations of the medius are smaller and thinner than the add. mand. ex. pars posterior they are derived from the same muscle. According to Edgworth (1935) the developmental phenomena in *Tropidonotus* show that the add. mand. ex. and int. are derivatives of the medius, i.e., that there was primarily only one add. mand. He also states that, after this division, the maxillary and mandibular roots of the fifth cranial nerve pass between the externus and medius. This last statement is borne out in *Thamnophis* if we assume the so-called add. mand. medius pars superficialis is the main body of the add. mand. medius. The main part of the mandibular root of the fifth nerve passes antero-lateral to this muscle on its ventral course into the mandible and the main branches are given off lateral to the outer surface of this muscle.

The action of both these muscles is undoubtedly adduction (in both medial and dorsal directions) of the mandible. The pars profundus, especially, is in a position to assist the intermandibularis musculature in approximating the two mandibles toward the mid-line after distension and may possibly have become separated off to assist in this action.

(iii) M. adductor mandibulae internus. The synonymy of this group shows a very definite trend to consideration of it as a part of the pterygoid musculature. This point has been discussed above, and will be considered no further here.

(*a*) pars anterior (referred to by Edgeworth as the add. mand. internus and by Adams as the pterygoideus anterior). One of the most significant points regarding this and the following muscle is that both their origins and their insertions are on potentially movable parts. Thus if pars anterior contracted, either protraction of the mandibular segment or retraction and holding of the maxillary segment, and more especially the maxilla, will occur depending on whether the cervico-mandibularis, in particular, contracts or relaxes.

Despite the varied nomenclature of this muscle, there seems little disagreement among the various writers as to its existence and general morphological characteristics—probably less than for any other muscle described in this paper. This might indicate a greater degree of stability in the function of this muscle as opposed to certain others. Certainly the muscle is well used, if we may judge at all by its relative size.

(*b*) pars posterior (referred to by Edgeworth as the accessorius and by Adams as the. pterygoideus posterior). Here, as in the pars anterior, both origin and insertion are on movable regions. The action of this muscle, on contraction, must be to bring the posterior tips of the maxillary and mandibular segments together, although, excluding other actions, protraction of the mandible to a very limited extent may be accomplished. If the protractor pterygoidei is relaxed, and the cervico-mand. is in contraction, abduction of the maxillary segment will occur to a limited extent; but if the protractor pterygoidei contracts, the main movement will be the result of this muscle's action and the pars posterior ends of the pterygoid and mandible together.

It is worthy of note that this muscle is not present in *Typhlopidae* or *Boidae* (according to Edgeworth) and is thus probably a feature of the higher forms only.

2. THE CONSTRICTORES DORSALIS. Edgeworth has concluded that the primary form of this dorsal division of the masticatory muscle plate in Sauropsida was a muscle passing from the pleurosphenoid (or sphenolateral plate) to the palatoquadrate and pterygoid bones. This, in the Ophidia, has become separated into five fasciculi inserted into the vomer, palatine, pterygoid, and quadrate. The synonymy of these muscles does not show the same degree of variation as in other groups, although different concepts of homologies have led to rather different nomenclatures.

From the anterior backwards, the group includes:

(i) M. retractor vomeris (vomero-sphenoidalis, of Adams). The action is chiefly to hold and retract the premaxillary segment.

(ii) M. retractor pterygoidei (pterygo-sphenoidalis anterior of Adams). The name applied to this muscle, presumably based solely on function, may seem rather confusing as the muscle has no attachment to the pterygoid anywhere along its course, but is inserted entirely on the palatine. However, inasmuch as both palatine and pterygoid are closely united in a seemingly immovable union, the retraction of the palatine must certainly be carried to the pterygoid, and as the relations of this bone are far more complex, the name retractor pterygoidei has been used by Edgeworth.

The action, of course, is retraction of the more medial half of the maxillary segment, involving the palatine and pterygoid. This movement will be transmitted through the ectopterygoid to the maxilla, but, as may be seen from the relative position of these bones, retraction of the pterygoid does not necessarily involve with it the same amount of retraction of the add. mand. int. pars anterior previously described. Adduction of the posterior end of the maxilla would precede retraction in any case.

(iii) M. levator pterygoidei (pterygo-parietalis of Adams). The action of this muscle is partially, though not entirely, expressed in the name applied. Apart from the fact that contraction raises not only the pterygoid but the entire maxillary segment, and to a certain extent the mandibular via the posterior connection, the factor of protraction of the pterygoid, palatine, and associated bones of this segment must be considered. This muscle undoubtedly enables the maxillary segment, with its three sets of teeth, to disengage and move forward in preparation for a new hold on the object being swallowed.

(iv) M. protractor pterygoidei (pterygo-sphenoidalis posterior of Adams). The action is protraction of both the maxillary and the mandibular segments and almost certainly abduction of their anterior ends (involving especially the dentary and maxilla). The contraction of this muscle would supplement, then, the action of levator pterygoidei.

(v) M. protractor quadrati (sub-occipito-quadrato-mandibularis of Adams). The action is protraction of the mandibular segment especially, and abduction or spreading of the anterior tips of the mandibles. The name for this muscle used by Edgeworth seems rather unsuitable, at least in these forms. No attachment of the muscle was found at any point on the quadrate, and although it is true that its contraction would protract the quadrate to a limited extent, this would seem to be a secondary result of the more functionally significant protraction of the mandible itself.

The small size and relative weakness of this muscle possibly indicates that its protractive function, at least, has been taken over to a great extent by the more stable add. mand. int. pars anterior.

II. *The Intermandibular Muscles*

Presuming that the following homologies are correct, and that all the muscles described as being in this group actually have separated from the primordial intermandibular sheet, there has been, in genus *Thamnophis*, a greater division of the intermandibularis sheet than has yet been described for any other ophidian genus. Edgeworth has stated the division to be into two or three portions, and Adams, in describing *Natrix cyclopion*, has described four muscles which correspond to portions of the intermandibularis. Gnanamuthu, basing most of his brief description of the "transverse muscles" on the seemingly radical *Helicops schistosus*, has made use of a very confusing terminology, giving no consideration to groups, homologies, or possible origins. Because of this, and the different appearance of the muscles shown in his text figures, it would be difficult to homologize the muscles described by him with the intermandibularis divisions described here. No attempt, therefore, at a synonymy of these muscles with those used by Gnanamuthu will be made.

It is, of course, entirely possible that, considering the apparent variations, the muscles described here are not homologous with individual muscles of other genera or families. The type of division of the primordial sheet found in genus *Thamnophis* may be peculiar to this and a few other genera. It seems that a great deal more descriptive and developmental anatomical work must be done before a comparative study of this muscle group in the Ophidia can yield any conclusive results.

Edgeworth divides the group into a maximum of three muscles: intermand. anterior, transversus branchialis, and intermand. posterior. It is assumed, for reasons outlined below, that both the intermand. anterior and posterior have become subdivided into entirely separate muscles in this genus. It should be noted that the continuous raphe of insertion of the greatest part of the mass is suggestive of their origin from a single muscle sheet. It is the origins, far more than the insertions, that have changed.

(i) M. intermandibularis anterior. Adams (1925) describes the intermandibularis muscle in *Natrix cyclopion* as follows: "The intermandibularis or symphysial muscle originates on the inner side of the tips of the mandibles and extends to a median line, it splits, the posterior slip spreading to join the mass of fascia in this region of the throat." The insertion of this muscle in *Natrix cyclopion* corresponds closely, then, with the insertion of the two portions of the intermand. anterior described here. This condition of partial division of the intermandibularis anterior would certainly suggest that in genus *Thamnophis* the division has become complete, and the muscle formerly represented by a single intermandibularis is here represented by a double muscle, the result of the complete division of the seemingly more primitive structure. The condition of *Natrix*, then, would seem to be a phylogenetic stage in this particular specialization.

With regard to their most obvious relationships, the muscles have been termed pars anterior and pars posterior of the intermandibularis anterior.

The function of these two muscles is direct adduction of the tips of the mandibles. An indirect function is undoubtedly elevation of the glottis, during swallowing, by the upward force exerted by these muscles when stretched by the spreading of the mandibles.

The portion of the pars anterior attached to the buccal floor probably has the same functional significance as that described below for the transversus branchialis.

(ii) M. transversus branchialis. This muscle has been referred to by only a few writers. Edgeworth (1935) refers to it merely as "a more dorsally situated portion" of the intermandibularis musculature, not present in all Ophidia. Adams makes no reference to it in any of his work.

The paucity of information regarding this muscle makes it advisable to consider here only the condition in *Thamnophis*. The muscle has lost entirely its origin on the mandible, although its raphe of insertion remains continuous with that of the greater part of the intermandibularis insertion. This presumably new origin on the buccal membrane has a definite functional significance. From a superficial examination of the resting oral cavity of the snake, it will be noted that the entire buccal membrane is thrown into folds, which, when stretched out, could increase the area of the membrane by at least two times. If there were no muscle attachments to this membrane, the folds would be a considerable source of trouble in swallowing a large object, but if there is some means of evenly distributing this membrane over the greatly expanded surface of the distended oral cavity, a considerable advantage will be gained. This muscle, and a few posterior fibres of the intermand. ant. pars anterior which are directed dorsally to insert on this membrane, will obviously tend to pull on the floor of the buccal cavity when the mandibles are expanded, thus preventing an abnormal distribution of the membrane.

Another probable action of this small muscle is in changing the fibre direction of the contracting genioglossi, especially their lateral heads. When the mandibles are not distended, as would be the case when the lingual apparatus is in use, contraction of this muscle would undoubtedly help to bind the genioglossi of each side to the hyoglossus muscle band.

(iii) M. intermandibularis posterior. In genus *Thamnophis* two entirely separate muscles appear which the writers have presumed to have arisen by a division of a more primitive intermand. posterior. These two muscles have been termed the pars anterior and pars posterior of the intermand. post. with reference to their most obvious relationships. Adams (1925) describes two muscles, the adductor medius and adductor posterior, in *Natrix cyclopion* which correspond respectively to the two muscles described here. Edgeworth has described the intermand. post. as arising from the middle or hinder part of the jaw-and passing inwards and forwards to the middle line or symphysis, or crossing its fellow to the opposite jaw. Evidently, in *Thamnophis*, both the middle *and* hinder ends of the jaw have been utilized as origins for the two supposed divisions of this muscle.

The individual action of the pars anterior of each side, and to a lesser extent the pars posterior, would almost certainly be to protract the mandible of that side. An additional action, especially attributable to the pars posterior, would be that of adduction of the more posterior part of the mandibular segment after distension in swallowing.

This specialization may quite possibly have arisen to suit these functions, especially that of the pars anterior in protraction. From the apparently complete division of this group in *Natrix cyclopion*, where the intermand. ant is only partially split, we may postulate that this division is the older, phylogenetically, of the two and has consequently become more stable as regards function.

THE HYOID MUSCLES

Perhaps because of the comparative conspicuousness of most of the muscles of this group, all, with the notable exception of con. colli., have generally been covered in any work done on ophidian head musculature. As usual, however, little attempt has been made to consider them in a comparative light, and consequently we find a very large synonymy in connection with most of them. The functions ascribed (apart from those of the con. colli) are commonly given under an indiscriminate label "depressor mandibulae" with no consideration of other possible functions. The very complex neuro-costo-mandibularis, although fairly well described in some cases, has presented a problem in homology which no one has seemingly cared to discuss. Thus it may be seen that, although in general three of these four muscles are reasonably well known, their relationships are little understood.

I. *Constrictor Colli*

(i) M. constrictor colli. This muscle was described by Duvernoy (1832) in *Coluber natrix* as the "Peau cier du cou." Edgeworth states that it is present also in *Python* and *Tropidonotus* but has not been described in other genera. Adams makes no reference to any such muscle in *Natrix cyclopion*, nor does Gnanamuthu in *Helicops*. It is possible that this muscle, of extremely thin structure and very superficial, may have been overlooked by some of the other workers, or it is more probable that this muscle is being lost in the Ophidia, this process having been completed in some genera. This muscle, which is very well developed in long-necked forms such as the turtle, may quite logically be no longer essential to forms such as the Ophidia. The presence in the members of the genus *Thamnophis* of two stages in what we might term the phylogenetic degeneration of the muscle may lend support to this argument.

II. *Depressores Mandibulae*

(i) M. occipito-quadrato-mandibularis. There has been a general tendency to consider this muscle as part of the "temporalis" or as the digastric. However, it can have no possible homology with either of these.

Edgeworth (1907) suggests that the otherwise parietal origin of the depressor mandibulae (referring to this muscle) on the quadrate in Ophidia is a new development. If this change has taken place within the Ophidia, as it presumably has, we may expect to find transitional stages where the origin is on both the occipital region and the squamosal and quadrate. Indeed, Edgeworth (1935) has described the usual origin of this muscle as by two heads—one from the quadrate and the other from the occipital region of the skull. He further states that in *Tropidonotus* the muscle arises only from the quadrate, whereas in the more primitive *Typhlops* it arises from the parietal. The phylogenetic stages of this change in the origin of the muscle are clearly brought out in the three members of genus *Thamnophis* studied in this work. It has been noted that in *sirtalis* and *elegans* the entire origin is on the quadrate, while in *ordinoides* it is almost entirely on the occipital region. It may be presumed, then, that *ordinoides* has not proceeded as far in this line of specialization as the other two species. Why these changes have proceeded further in *elegans* and *sirtalis* presents a rather difficult problem. There may be some significance in the fact that the occipital origin is limited to the terrestrial form, and that the further stage is found in both so-called aquatic forms.

In general, the concerted action of these muscles will be depression of the mandibles, at the same time separation of their tips to a certain extent. Used singly, they aid in the swallowing process, their action on the mandibular segment corresponding very closely to the action of the levator pterygoidei on the maxillary segment—that of releasing the old grip and protracting for a new, more anterior grip.

(ii) M. cervico-mandibularis (the depressor mandibulae of Adams, 1915, 1925). At least in genus *Thamnophis*, the commonly ascribed action of this muscle as a depressor of the mandible seems to be to a greater extent replaced by the function of opposing the chief protractor of the mandibular segment, the add. mand. int. pars anterior, thus enabling the retraction of the entire maxillary segment. Disregarding the add. mand. int. pars anterior action, the effect of contraction would be mainly retraction of the mandibular segment and possibly, to a slight extent, depression of the mandibles. However, the function of reversing the action of the add. mand. int. pars anterior is probably its most important function.

(iii) M. neuro-costo-mandibularis (Adams, 1925). This muscle, with its three heads of origin in genus *Thamnophis*, is by far the most complex individual muscle in the groups studied here. Though recognized and described by most writers, it has been treated only from a descriptive standpoint and no adequate information has been noted regarding the possible origins of this muscle. There has been a general tendency to break this muscle down into two parts: the neuro-mandibularis and the costo-mandibularis, corresponding to the first two heads of origin described here. But nowhere in the literature has there been any reference to a third head of origin from the hyoid apparatus. Edgeworth, however, states that the medial fibres of this muscle are inserted into the ceratohyal and the lingual sheath. To describe this attachment of fibres of the muscles in *Thamnophis* as an insertion is hardly logical, and therefore it is referred to here as the hyoid head of the muscle. Although proof is lacking, there is very little doubt that these fibres, which have arisen from the hyoid and hyoglossus sheath, represent the geniohyoid muscle which has become incorporated into the medial anterior part of the neuro-costo-mandibularis. Edgeworth (1935) has described the geniohyoideus as passing from the anterior end of the jaw to the basihyal.

An examination of the earlier work on ophidian musculature tends to support this conclusion. For instance, Gnanamuthu (1937) describes the geniohyoideus in *Helicops* as having origin on the mandible and insertion on the basihyal and ceratohyals. He, however, gives no description of any neuro-costo-mand. muscle or any relationships of the geniohyoideus which, in his figure of *Helicops*, looks very much like the medial half of the neuro-costo-mand. in these forms. Adams, in *Natrix cyclopion*, has described a separate mylohyoid muscle, which from his figures is the exact homologue of the most anterior and medial portion of the neuro-costo-mand. in *Thamnophis*. This muscle he describes as arising from the ventral side of the dentary, anterior to the insertion of the neuro-costo-mand. and extending posteriorly to be inserted on the anterior fourth of the hyoid bone. (This mylohyoid is synonymous with the geniohyoid of Edgeworth according to his own synonymy.) Thus there seems little doubt that this head of the neuro-costo-mand. represents the former insertion of a geniohyoideus.

It is generally accepted that the origin of a geniohyoid is on the mandible and its insertion is on the hyoid. However, as previously indicated, it seems entirely illogical to presume that this attachment of fibres to the hyoid is an insertion when the entire muscle, whatever its original components, now inserts in one continuous sheet on the mandible. It is difficult to see how one portion of a single connection of muscle to bone can be origin and another insertion. Perhaps this indicates again the rather arbitrary nature by which origins and insertions are assumed. While this band of fibres remains separate from the neuro-costo-mand. as a geniohyoideus, it is only correct to conform to established procedure; but in the case as we find it in genus *Thamnophis* the origin and insertion of this head of fibres must be reversed or a very confusing picture of the relationships of this entire muscle will ensue.

Regarding the original neuro-costo-mand., Edgeworth has described no developmental stages, merely including both heads under the depressor mandibulae portion of the hyoid musculature—a portion which has separated off from the dorsal part of the anterior edge of the hyoid muscle plate. This origin seems quite reasonable for the vertebral head, but position and relationships of the costal head, especially to the ceratobranchials and the closely apposed rectus cervicis musculature, suggest a possible origin from the rectus cervicis mass of the hypobranchial spinal musculature, or from the branchial musculature. A further study of both development and nerve supply should prove interesting in clearing up this point.

The actions brought about by contraction of this muscle are probably equally as complex as the muscle itself. If each head of the muscle can act independently, there will be, of course, a much more diverse series of actions than if the muscle can contract only as a unit. It may be assumed, with reasonable assurance, that the prime function of the muscle is as a depressor and retractor of the mandibles. It would seem also to approximate the mandibles, although this seems contradictory to what we might expect. The hyoid head is in a position to oppose the retractive action of the cervico-hyoideus on the basihyal and probably acts partly in this capacity.

THE HYPOBRANCHIAL SPINAL MUSCLES

I. *The Rectus Cervicis Group*

This group has been very inadequately treated in the literature. Even Edgeworth makes only a passing reference to its existence and Adams describes only one of its derivatives. The muscles which Gnanamuthu refers to as sternothyroideus and omohyoideus in *Helicops schistosus* may possibly be members of this group although he makes no statement to this effect, merely describing them as muscles which have lost their lacertilian insertion on the sternum and scapula to become inserted on the skin. As has been mentioned above, there is a possibility, from superficial evidence, that a portion of this group may have become incorporated into the neuro-costo-mand.

(i) M. cervico-hyoideus (Hoffmann, 1890). This muscle may be the homologue of what Gnanamuthu describes as sternothyroideus and omohyoideus in *Helicops*. Its action is problematic, but the muscle covers the ventral surface of the hyoglossus and probably has an important function in anchoring the hyoid apparatus against the effects of contraction of the hyoid head of the neuro-costomand. The effect of its contraction on the skin may be of some functional significance in locomotion.

II. *The Geniohyoid Group*

The probable fate of the geniohyoid muscle in this group has already been discussed and no further consideration will be given to it here.

Edgeworth (1935) states that the genioglossus and geniotrachealis are developments from the anterior end of the geniohyoideus and that the hyolaryngeus is a development of the posterior end of the hyoglossus. Gnanamuthu (1937) describes the geniotrachealis and hyolaryngeus as the "tracheal and buccal" divisions of a "genioglossus ventralis" if our synonymy is correct.

(i) M. genioglossus. The action of this muscle as a protractor of the tongue will be considered along with the discussion of the hyoglossus muscle below.

(ii) M. hyoglossus. This muscle, though actually only the chief muscular portion of the tongue, is more often referred to as the tongue, probably because of its bifid external part. Its anterior end is surrounded by the tongue sheath, a sheath containing certain intrinsic tongue muscles, and further back it is enveloped on three sides by sheath-like genioglossi.

The action of the genioglossus on this muscle, and its consequent action on the ceratohyals, make up, in the main, the extremely specialized mechanism for tongue protrusion and retraction in snakes. Contraction of the genioglossi protracts the hyoglossi directly anteriorly through the tongue sheath, which acts to direct the course of this long flexible "tongue." Theoretically, the tongue could be protracted about one-third of its length, but it is doubtful whether the genioglossi could contract to this extent. The retraction of the "tongue" is peculiar in that the hyoglossi cannot play their usual role of retractores linguae because their origins are not firm, and furthermore they are themselves the main body of the tongue. Gnanamuthu has described the mechanism of retraction as consisting of the elastic recoil of the ceratohyals, which, in protraction of the hyoglossi, have become flexed. It seems rather improbable that this alone accounts for the retraction of the hyoglossi. There may be a band of elastic tissue between the posterior tips of the hyoglossi and of the ceratohyals which may act as a retracting force. Certainly a histological study of the concentrations of elastic fibres in the tissues of this area would suggest a more definite answer to this problem.

The intrinsic muscles of the tongue, the actions involved in its sensory adaptation, and the mechanisms behind these actions are all beyond the planned scope of this study.

(iii) M. geniotrachealis (maxillo-laryngeus of Adams, 1925). The only apparent action of this stout muscle band seems to be that of protraction of the trachea. This is probably of considerable functional significance in enabling the animal to breath while swallowing a large object.

This muscle has been included in almost every work which has dealt with the ventral musculature. It seems to be a reasonably stable muscle whose function must be quite essential to the animal's well-being.

Before concluding this section of the discussion, a brief résumé of the interrelated actions of the muscles used in food getting by one of these animals may be pertinent. As an approach to this, let us assume a hypothetical case of an engagement between a member of genus *Thamnophis* and a common field mouse. We shall presume that all preliminary sensory behaviour, with its use of the lingual apparatus, has ceased and that the snake is in the act of striking at its prey. The mandibles are depressed by the concerted actions of the occ.-quad.-mand. and neuro-costo-mand. muscles. Probably abduction of the anterior tips of the mandibles, involving the prot. quad., occ.-quad.-mand., and cerv.-mand. muscles, has also taken place and also protraction of the entire mandibular segment, involving action by the partes anterior and posterior of the add. mand. int., the prot. quad., and the pars anterior of the intermand. post.

With the head, preferably, of the prey in its mouth, the jaws are clamped shut by action of the entire add. mand. externus and medius groups and the retractor vomeris is contracted to hold the premaxillary segment. Distension of the oral cavity is accompanied by spreading out of the buccal membrane, assisted by the trans. branch. and intermand. ant. pars anterior. The process of crawling over the wriggling prey now begins. There are three possibilities here: (*a*) release and protract the entire upper or lower teeth as a unit; (*b*) release and protract the maxillary and mandibular segments of each side as a unit; (*c*) release and protract each individual segment separately.

In absence of evidence, it will be assumed that (*c*) takes place, and the movements on one side only will be treated. Let us assume that the first region to disengage is the right maxillary. To accomplish this, the contraction of the lev. pterygoidei will be brought into play, and when the teeth are disengaged, continued action by this muscle, supplemented by action of the prot. pterygoidei will protract this segment to a new, more anterior, position. The retractors of this segment will then contract, involving the ret. pterygoidei and add. mand. int. pars anterior (if the cerv.-mand. contracts), and their effect will be to drive the backward directed teeth into the prey's back and drag it further into the mouth. When this new hold has been gained, the mandibular segment of the same side will disengage its grip. This will involve a measure of relaxation on the part of the adductores of the mandible, and the actual disengaging movement will arise from action of the usual depressors of the mandible. Protraction will occur with this release through the action of the add. mand. int. pars anterior, which is now pulling both ways, the cerv. mand. (relaxing to allow the add. mand. int. pars anterior to affect the mandible), the prot. quad., the occ.-quad.-mand. (probably the most effective muscle in accomplishing this movement of disengaging and protracting the mandibular segment, which corresponds closely to the action of the lev. pterygoidei on the maxillary segment); and the intermand. post. As soon as protraction has occurred, the adductors and retractors of the mandibular segment will contract, involving the add. mand. externus and medius, the neuro-costo-mand., and the cerv.-mand., and a new hold will be gained in this region.

This may next be in the mandibular or maxillary region of the opposite side. The actual sequence is immaterial but the actions as described above are probably as they actually occur.

It must be remembered that, during this time, the prey itself is doing a considerable amount of wriggling, and the snake must wait an opportunity for the taking of these new holds. All the while, however, the prey is being subjected to a considerable strain due partly to the elastic tendency of the distended oral cavity but more especially to the contraction of the intermandibularis musculature, so that the prey is slowly suffocated. As the prey becomes more quiescent, the "crawling" movements will proceed more rapidly, though in the first few minutes after the initial engagement the entire effort on the part of the snake must depend chiefly on the adductors and retractors of the three segments.

There being no protraction to the premaxillary segment, it is assumed that this becomes disengaged as the maxillary segments take new holds, and its prime function is probably accomplished in the initial effort of "hanging onto" the prey.

At the moment of disengaging and protracting of one segment, the retractors of the other three segments probably exert an extra effort so that a maximum advantage may be obtained with the new hold.

The elevation and protrusion of the glottis by the geniotrachealis and upward force of the intermand. ant. is probably of great importance in enabling the animal to breathe while swallowing.

Possible Phylogenetic Relationships and Functional Adaptations within the Three Species

A consideration of the three species described in this paper is now pertinent in an attempt to relate the differences found between them to features of their present environment or to their stage in phylogenetic development.

Fitch (1940) has provided an excellent ecological study of the *Ordinoides* Artenkreise of garter snakes, with a comparison and contrast to the *sirtalis* group. To this paper the reader is referred for information regarding food habits, ecology, etc., of the snakes studied here.

The most obvious feature to be noted on a superficial examination of the heads of these three species is the contrast in shape between the "terrestrial" and "aquatic" forms. A narrow, pointed head is apparently an adaptation to an aquatic habitat; a broad, shorter head is associated with the enlarged supralabial glands, supplying the needed lubrication for ingestion of terrestrial prey. In any case, we have noted a very close similarity between the jaw lengths of the two aquatic forms, and a rather marked contrast between the length of these and the short jaws of *ordinoides*. The difference in jaw length between *ordinoides* on one hand and *elegans* and *sirtalis* on the other has a pronounced effect on the appearance of the musculature, especially the adductor mass. The general compact and massive form of the adductores mandibulae and the short, stout quadrate in *ordinoides* indicate that these portions, at least, are put to greater use. This, however, seems to be the only functional adaptation which may be attributed to the food habits of the animals—apart from the effect of the "stream-lining" of the head or the general appearance of the musculature.

The radical variations noted in the origins of the occ.-quad.-mand. and the con. colli muscles among the three species are probably not due as much to the modification for different environments among the three forms as to the particular phylogenetic stage to which these individual species have developed, though this stage may have been governed by the needs of the environment. We have considered previously that the most primitive form of each muscle is represented by its most dorsal origin. Thus, with regard to the occ.-quad.-mand., which is apparently assuming a new origin on the quadrate in the Ophidia, we may assume that in *elegans* and *sirtalis* this line of specialization has been carried further than in *ordinoides*. Possibly there may be some functional value in the retention of the occipital origin in *ordinoides*—perhaps in supplementing the holding function of the ligament connecting the quadrate and skull.

The same may be said of constrictor colli, although it apparently retains its primitive condition only in *sirtalis*. This would seem to indicate a phylogenetic advance by the *ordinoides* species over *sirtalis*. There seems, again, to be little explanation for the retention of the dorsal fibres in *sirtalis*. It was noted that, in some specimens, the dorsal portion of the muscle was less developed than in others, thus indicating that *sirtalis*, too, is losing at least this portion of the muscle.

Disregarding the comparative condition of the constrictor colli in the three forms, it is very obvious that there is a far greater similarity between the "aquatic" forms of the two species than between the two phylogenetically related species. In fact, with the skin removed, it would be difficult to distinguish *sirtalis* from *elegans* in a superficial examination, while a specimen of *elegans* and a specimen of *ordinoides* could be very easily distinguished.

CONCLUSIONS

1. These species of genus *Thamnophis* appear to be unique among the Ophidia so far studied, in the following features:

(*a*) The M. adductor mandibulae medius has become divided to form an outer pars superficialis and an inner pars profundus.

(*b*) The M. intermandibularis anterior has become divided into a pars anterior and a pars posterior.

(*c*) The M. intermandibularis posterior has become divided into a pars anterior and a pars posterior.

(*d*) The M. geniohyoideus has become incorporated into the anterior medial portion of the M. neuro-costo-mandibularis with a corresponding reversal of its former origin and insertion.

(*e*) Two lateral horns, the first and second ceratobranchials, are appended to the anterior ends of the ceratohyals.

2. The costal head of the M. neuro-costo-mandibularis is probably derived from the hypobranchial spinal or branchial musculature rather than from the hyoid sheet.

3. Within this group of three races, the condition of muscles showing intermediate phylogenetic stages, particularly in the hyoid

group, is indicative of their instability and transitory existence in that stage. It is not possible to correlate the rate of these phylogenetic changes in all the unstable muscles of each race and thus place one race higher in the phylogenetic scale than another, on this basis. The rates of change among the unstable muscles of one race have apparently varied considerably, or possibly each was initiated at a different time. Thus, where one race may possess a more primitive condition in one unstable muscle than another race, another unstable muscle may be equal to or ahead of the corresponding muscle of the other race in specialization.

4. It is particularly significant to note that the races of two different species with similar habits have much greater similarity in cranial musculature than do the species more closely related but with different habits.

SUMMARY

A summary of the classification used, and derivations assumed for the muscles described in this paper, follows:

MANDIBULAR MUSCLES

Masticatory Muscles

- ADDUCTORES MANDIBULAE
  - M. adductor mandibulae externus
    - pars anterior
    - pars medius
    - pars posterior
  - M. adductor mandibulae medius
    - pars superficialis
    - pars profundus
  - M. adductor mandibulae internus
    - pars anterior
    - pars posterior
- CONSTRICTORES DORSALIS
  - M. retractor vomeris
  - M. retractor pterygoidei
  - M. levator pterygoidei
  - M. protractor pterygoidei
  - M. protractor quadrati

Intermandibular Muscles

- M. intermandibularis anterior
  - pars anterior
  - pars posterior

M. transversus branchialis
M. intermandibularis posterior
  pars anterior
  pars posterior

HYOID MUSCLES

Constrictor Colli

M. constrictor colli *

Depressores Mandibulae

M. occipito-quadrato-mandibularis *
M. cervico-mandibularis
M. neuro-costo-mandibularis (vertebral head)

HYPOBRANCHIAL SPINAL MUSCLES

Rectus Cervicis Group

M. cervico-hyoideus
M. cervico-quadratus
M. neuro-costo-mandibularis (costal head)

Geniohyoid Group

M. genioglossus
M. hyoglossus
M. geniotrachealis
M. hyolaryngeus
M. neuro-costo-mandibularis (hyoid head)

A summary, on the basis of probable function, of the muscles described follows:

(1) to adduct mandibles
  M. add. mand. externus
  M. add. mand. medius
(2) to abduct (tips) of mandibles
  M. protractor quadrati
  M. occ.-quad.-mandibularis
  M. cervico-mandibularis
(3) to protract mandibles
  M. add. mand. int. pars anterior
  M. add. mand. int. pars posterior
  M. protractor quadrati
  M. intermand. post. pars anterior

---

* Show marked variation among the three species described indicative of their unstable nature.

(4) to retract mandibles
M. neuro-costo-mandibularis
M. cervico-mandibularis
(5) to adduct (tips) of mandibles
M. intermand. anterior
M. intermand. posterior
(6) to depress mandibles
M. neuro-costo-mandibularis
M. occ.-quad.-mandibularis
M. cervico-mandibularis
(7) to retract premaxillary
M. retractor vomeris
(8) to retract maxillary
M. add. mand. int. pars anterior
M. retractor pterygoidei
(9) to elevate maxillary
M. levator pterygoidei
(10) to protract maxillary
M. protractor pterygoidei
M. levator pterygoidei
(11) to fix hyoid (basihyal)
M cervico-hyoideus
M. neuro-costo-mandibularis
(12) to protract "tongue"
M. genioglossus
(13) to protract trachea
M. geniotrachealis
(14) to retract trachea
M. hyolaryngeus
(15) to spread buccal membrane
M. intermand. ant. pars anterior
M. transversus branchialis

REFERENCES

ADAMS, L. A. (1915). A memoir on the phylogeny of the jaw muscles in recent and fossil vertebrates. Ann. N. Y. Acad. Sci 28: 51–166, pls. 1–13.

———(1925). Head muscles in *Natrix cyclopion*. J. Morph. 41: 159–77, pls. 1–2.

D'ALTON, E. (1834). Beschreibung des Muskelsystems eines Python bivitattus. Archiv. f. Anat. u. Physiol. u. wissensch. Med. (Johannes Müller) 7: 346–64; 10: 432–50; 12: 528-43.

DE BEER, G. R. (1937). The development of the vertebrate skull. Oxford. Pp. 1–515, pls. 1–143.

DUGES, A. (1827). Recherches anatomiques et physiologiques sur le déglutition dans les Reptiles. Ann. de Sci. nat. **12**: 337–95, pls. 46.

DUVERNOY, D. M. (1832). Mémoire sur les caractères tirés de l'anatomie pour distinguer les Serpents venimeux des Serpents non venimeux. Ann. de Sci. nat. **26**: 113–60, pls. 5–10.

EDGEWORTH, F. H. (1907). The development of the head-muscles in *Gallus domesticus* and the morphology of the head-muscles in the Sauropsida. Quart. J. Micr. Sci. (London) **51**: 511–56, 39 figs.

———(1911). On the morphology of the cranial muscles in some vertebrates. *Ibid.* **56**: 167–316, 100 figs.

———(1935). The cranial muscles of vertebrates. Cambridge. Pp. 300, 841 figs.

FITCH, H. S. (1940). The Ordinoides Artenkreise of garter snakes. Univ. Cal. Pub. Zool. **44**: 1–133, pls. 1-7.

GNANAMUTHU, C. P. (1937). A comparative study of the hyoid and tongue of some typical genera of reptiles. Proc. Zool. Soc. London B**107**: 1–63, 42 figs. in text.

HAGER, K. P. (1906). Die Kiefermuskeln der Schlangen und ihre Beziehungen zu den Speicheldrüsen. Zool. Jahrb., Abt. f. Anat. u. Ont. der Tiere **22**: 173–224, pls. 10-14.

HOFFMANN, C. R. (1890). Klassen und Ordnungen des Tier-Reichs, Bd. **6**, Abt. III: 1448–53.

LAKJER, T. (1926). Studien über die Trigeminus—Versorgte Kaumuskulatur der Sauropsiden. Kopenhagen: C. A. Reitzel. Pp. 154.

PHISALIX, Mme. M. (1914). Anatomie comparée de la tête et de l'appareil venimeux chez les serpents. Ann. de Sci. nat., N. S., **19**: 1–114.

VERSLUYS, J. (1912). Das Streptostylie-Problem und die Bewegungen im Schädel bei Sauropsiden. Zool. Jahrb., Sup. 15, **2**: 545–716.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLV : SERIES III : JUNE, 1951
SECTION FIVE

Resources of Arctic and Subarctic Seas*

M. J. DUNBAR
Presented by N. J. BERRILL, F.R.S.C.

IT has become the fashion, quite recently, to develop the north. Institutes, new government departments, discussion groups, have been formed to consider, assist, and effect the study and development of the arctic and subarctic regions—all in the past five years or so. To those of us who were interested in these problems many years before the present outburst of energy, this sudden preoccupation with the north seems over-enthusiastic and somewhat late. Over-enthusiastic, because claims are being made concerning possibilities in the north which certainly are founded upon very little observation; and late, because if the study of the north had been made steadily over the past fifty years we would have established the habit of northern research by this time, and would not have to be over-enthusiastic to stimulate interest and loosen the purse-strings.

As usual, the present trend has followed the land-bound habits of human beings, and there is an overwhelming emphasis upon the land rather than on the sea. It is true that the resources of the greatest potential value in dollars and cents, minerals, lie in the land, but it should not be forgotten that for more than a thousand years it has been upon the sea and not upon the land that man has relied for his support in northern parts of the world. The traditional Eskimo way of life is one of hunting, mostly on salt water. The Norsemen's colonization of west Greenland some nine hundred years ago was made possible largely by the resources of the sea, and the same is true of the Danish development in Greenland today. Furthermore, the first commercial interests in the north lay in whaling and sealing and in fishing, not in prospecting or in mining.

We have destroyed, at least for a time, the whaling industry in the north; we seem to be fast doing the same thing in the south. The sealing conditions are not the healthiest possible today, owing to the lack of a unified code of behaviour among the nations concerned. These are serious matters, both for the Eskimo population and for ourselves as civilized people. I believe that the time has come to take stock of these marine resources of the north, not in order to develop them ultimately in commerce, but simply in order to provide, for Eskimo development, a sound basis for the Eskimo economy. A sound Eskimo economy, based on the local resources, would be a great saving to our pocket-book; and it would be of immense value when the time comes, as it very soon will, to integrate the activities of the Eskimo population with our own in the north.

---

*Presented Wednesday, June 6, in the Oceanographic Session arranged by the Canadian Committee on Oceanography.

The traditional Eskimo hunting economy was largely dependent upon the sea. It was, moreover, dependent mainly upon the mammals of the sea, the seals and the whales. This was because the habitat and migration routes of the Eskimo had long been the shores of the arctic region, not the subarctic. The arctic waters are, or were, rich in mammals, but not in fishes. It is in the subarctic waters that fisheries can be, and have been, developed, but we may observe that even when the Eskimos came down into subarctic marine regions, where the development of considerable fisheries was possible, they still did not develop them until the arrival of the white man brought help and encouragement. The Eskimos are a very conservative people, and do not lightly turn from the ways of their forefathers to new patterns of economy—except of course where obvious rewards are immediately forthcoming, as in the sale of furs or souvenirs to the white man. In west Greenland, a markedly subarctic area, it was the Danes who developed the present native fishery; on the Labrador coast the great wealth in codfish and other species was not seriously touched before the arrival of the Moravians in the eighteenth century (Tanner, 1947). Indeed, it may well be supposed that a fishery of any size cannot be maintained without the help of civilized technology.

It is to this important difference between the arctic and subarctic marine regions that I wish to give special attention here. The delimitation of the arctic from the subarctic on land has been attempted by many students, using a variety of criteria; but no single criterion, or set of criteria, has so far been able to satisfy everyone. Furthermore, it is quite clear from the dividing limits so far published that the authors consider the sea of no importance, for once their limiting lines leave the land and begin to cross salt water they make no sense at all. They are apt, for instance, to depart from Belle Isle or Hamilton Inlet in a straight line for Cape Farewell, thus relegating to the arctic not only the Atlantic water in the west Greenland current, but even the obviously non-arctic water of the Labrador Sea.

In the marine environment, the delimitation of the two regions is much simpler, but it must be done for the sea only, without reference to the terrestrial conditions. If we define the "arctic" as those parts in which the water, or at least the surface layers (100-200 metres), is of polar origin (from the polar sea); the "boreal" as those parts of the temperate northern hemisphere in which the sea water is of purely Atlantic or Pacific origin; and the "subarctic" as those parts in which both kinds of water are found in the upper layers, then we have a sensible and indeed obvious system in which the lines of delimitation, although they may change position somewhat from season to season and from decade to decade, are fairly easy to draw at any given time. The fact that they change in position is indeed a virtue, since they thus reflect a dynamic and not a static system. Such a system of delimitation has already been published (Dunbar, 1951).

As a rule these three water types (arctic, boreal, and mixed) can be distinguished quite easily by physical and chemical means alone. The arctic water is very cold, usually below 0° C., and of low salinity, always below 34 ‰ and often below 33 ‰. The Atlantic water is much warmer, above 4° C. at least, and of higher salinity, always above 34 ‰ unless greatly influenced by coastal drainage. The subarctic water lies naturally between these two in both parameters. Thus the west Greenland water, of 2 to 3° C. and 33 to 35 ‰, is obviously subarctic. But there are occasions when these criteria are not adequate, and in such cases we have to call upon living indicators which are known to be limited to non-arctic water, and are therefore, in the extreme limits of their distribution, useful as indicators of the northern limits of the subarctic. These indicators are necessary, for instance, to assess the nature of the water of Ungava Bay. It may be argued that if these indicator species (Atlantic cod and Atlantic salmon are two good examples) are restricted to water of boreal or subarctic parameters, then how is it that they are also found where the significant values of these physical parameters are not apparent? The answer is, I believe, that the characteristics of temperature and salinity may be obscured by local conditions of freshwater drainage and vertical turbulence, which disturb our observations more than they disturb the codfish and the salmon.

The arctic marine region is rich in mammals but poor in fishes. I do not believe that this is caused specifically by differences in productivity between arctic and other water (although such differences do exist), so much as by the fact that the mammals are homoiothermous and the fishes poikilothermous, and hence that the process of recolonization of the arctic water since the last glaciation presented less difficulty to the former than to the latter. It is to be expected that within the next few thousand years, or tens of thousands of years, the fishes of the present subarctic, or their evolved descendants, will have invaded the arctic waters. At present, however, it seems that for most fishes the very low temperatures of arctic water present a condition which renders growth and reproduction impossible. It is worthy of notice that the Cottidae, better represented in arctic waters than any other family of fishes, are generally considered to have dispersed in postglacial times from a Bering Sea refuge, where they should have been, from the start, acclimatized to low temperatures; and that the Coregonidae have come to North America from a Siberian refuge. Speculation is also legitimate here about the date of arrival in arctic water of the invertebrate groups which, with their lower organization and presumably greater lability, shorter generations, and greater breeding potential, might be expected to overcome low temperature and other barriers in a shorter time than other poikilotherms.

Be that as it may, there is no doubt (1) that the only fish of economic significance in arctic sea water is the arctic char (*Salvelinus alpinus*); (2) that the possibilities of important salt water fisheries are to be found in subarctic and not in arctic waters; and (3) that there is an obvious difference in the quantities of living matter produced in the two types of water, the greater production by far being in the subarctic. There has as yet been very little quantitative study made of this last point, largely because arctic marine research is still young enough to demand predominantly qualitative attention; but even without the use of quantitative methods for measuring plankton production (methods which are still open to considerable suspicion), the difference in the results of hauling plankton with ordinary open nets in arctic and subarctic water is most striking. Thus, throughout the open water season, the same plankton net, hauled for the same time, and with all conditions equal, will yield very much less plankton in the arctic water of the Baffin Island coasts than in the rich subarctic waters of west Greenland or Newfoundland, the ratio of the quantities being roughly between 1 : 3 and 1 : 6 or more, depending on the locality.

It is, so far as we know at present, only in temperature that arctic and subarctic waters differ significantly, at least in relation to this difference in productivity. It is therefore in the temperature difference that we should first look for the explanation of the phenomenon. Subarctic water is always water in which polar and non-polar water come together, and we have therefore to examine the histories of the two water types before junction or mixing occurs. The Atlantic water has no special characteristics, other than the higher temperature, to which we can point as relevant to the present discussion; but the arctic water has several.

The lower temperature allows high concentrations of dissolved gases, so that carbon dioxide and oxygen are present in abundance in polar water. This has been confirmed by empirical observation. Neither oxygen nor carbon dioxide, however, appear to be limiting factors in production in the sea generally, so that polar water is not exceptional in the possession of excess gases. The high concentrations, however, are important as indices of high potential production.

Neither phosphates nor nitrates are normally limiting factors in the sea (except possibly temporarily, in midsummer) so that their concentrations in arctic water are not immediately relevant to the matter in hand. Indeed, little study has been made of the concentration of these salts in the north. Measurements in west Greenland and in the Labrador, both subarctic regions, have shown normal levels. The only special condition which arctic water shows in this respect is the high population of sea-birds in spring and summer, which provides a mechanism for the immediate return of nutrient salts to the surface water, via the birds' faeces, without the necessity of vertical exchange of water. This is well demonstrated in the vicinity of breeding bird colonies in subarctic regions, where the water is often thick with diatom growth all summer long, in contrast to the surface elsewhere, which presumably becomes exhausted of nutrients in midsummer. This effect appears to be much less marked in arctic water, where apparently the growth is kept down by low temperature.

The long days of the northern summer most probably favour the growth of the phytoplankton, although there has been no definitive experimental work to show just what their precise effect is.

This high potential for the production of life in arctic water clearly is not realized until admixture with non-arctic water takes place, in the subarctic. The obvious inhibiting factor is the low temperature. Upon contact with the warmer Atlantic (or Pacific) water, this inhibition is removed, and the result is the great blossoming of life which has been described. The turbulent nature of subarctic water, with low stability, no doubt assists this process.

It has been pointed out, however, that these factors of potential production in arctic water (gases, phosphates, and nitrates) are seldom if ever considerable limiting factors in the life of the sea, and the question arises as to whether or not they can therefore explain entirely this great subarctic release of energy. Nor can it be said that the length of the summer days is the deciding factor, for the subarctic regions are not in equal latitudes. It may be, therefore, that there is some other character of arctic water which is perhaps of first importance in this matter, and I would suggest here that the possibility that this factor is one of the molecular structure of the water itself should not be overlooked.

I am aware that in discussing the molecular structure of water I am invading a field outside my own; it is also a field which is at present a controversial one, even within the circles of physicists and physical chemists who know most about it. The controversy, however, concerns the specific polymers found in water, rather than the very existence of the polymers themselves. It appears to be generally agreed that the most reasonable explanation of the anomalies of the behaviour of water, considered as consisting of uniform monohydrol (H_20) molecules, is that water in fact consists of polymers of the monohydrol in equilibrium; and that the state of the equilibrium depends in part upon the temperature of the water and in part upon its recent history. The disagreement enters when the proportions of the polymers present under given conditions, and the number of monohydrols within those polymers, are considered. This is not a point which concerns us here, and in discussing the relevance of the polymers to the production of life in the sea, it should be understood that when the term "trihydrol" is used here (the trihydrol being one of the more popular polymers), it is used simply to denote the largest aggregate commonly present in water, whatever it may finally turn out to be

In theory, the trihydrol (or other polymer) is at its greatest concentration in ice, and in the liquid medium is dominant in water recently melted from ice. It is at its minimum concentration in steam, and in water recently condensed from steam, in which the monohydrol dominates. Arctic water contains at all times, and especially in spring and summer, an unestimated large amount of water recently melted from ice, and therefore should contain a high proportion of the trihydrol (or other water polymer), even allowing for the presence of solutes, which appear to simplify the physical behaviour of water and which have even been supposed to break up the water polymers in high solute concentrations.

Some seventeen years ago experiments were done by Barnes (1932), Barnes and Jahn (1933), and by Harvey (1933), all showing that water recently melted from ice had a decidedly stimulating effect on the growth and multiplication of green cells (*Spirogyra*, *Euglena*, *Nitzschia*). In the case of *Euglena*, for instance, Barnes and Jahn (1933) found that the average increase in the number of cells in the condensed steam water, in experiments of ten to sixteen days' duration, was 32 per cent, while the increase in the ice water culture was 105 per cent. The pH of the two water types was the same, from which it was inferred that the CO_2 concentration was approximately the same in both. The effect has also been observed in cultures of *Bacterium coli* (Hegarty and Rahn, 1934); in the experiments in question air was bubbled through the water samples to avoid any effect from oxygen deficiency.

It is to be hoped that there will be evolved, in due course, a method of measuring the proportion of higher water polymers directly; so far, efforts in this direction have not been successful. In the meantime, there seems to be no better way of explaining the results of the experiments I have mentioned than by assuming that the polymers do exist, that their concentration is a function of the recent history of the water, and that they stimulate, in a manner not yet known, the growth and multiplication of holophytic cells. Barnes and Jahn (1934) suggested that this effect might help to explain the "richness of plant forms in arctic water." I believe that this effect may be very important, although clearly the question is one of an increase in the *potential* for production in arctic water rather than in the actual production. It is probable that one important contribution of arctic water to the subarctic, where the temperature inhibition is removed, is in fact ice water.

REFERENCES

BARNES, T. C. (1932). The physiological effect of trihydrol in water. Proc. Nat. Acad. Sci., 18: 136-7

BARNES, T. C. and JAHN, T. L. (1933). The effect of ice and steam on *Euglena*. Proc. Nat. Acad. Sci., 19: 638-40.

——— (1934). Properties of water of biological interest. Quart. Rev. Biol., 9 (3): 292-341.

DUNBAR, M. J. (1951). Eastern Arctic waters. Fish. Res. Bd. Can., Bull. no. 88.

HARVEY, H. W. (1933). On the rate of diatom growth. J. Mar. Biol. Ass., U. K., 19: 253-75.

HEGARTY, C. P. and RAHN, O. (1934). Growth retarded by freshly distilled water. J. Bacteriol., 28: 21-30.

TANNER, V. (1947). Outline of the geography, life, and customs of Newfoundland and Labrador. Cambridge: Cambridge University Press.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLV : SERIES III : JUNE, 1951
SECTION FIVE

The Action of Sodium Salicylate in Protein Dispersion

A. G. McCALLA
Presented by R. NEWTON, F.R.S C.

SODIUM salicylate in solution has been used as a protein dispersing agent by a number of investigators, particularly during the past twenty or twenty-five years. Some of the earlier results indicated that this salt caused denaturation of the protein. Anson and Mirsky for example (1931) reported that it altered or denatured albumin, and scattered reports of denaturation effects have subsequently been published. The basic thesis of this paper, however, ignores the question of denaturation as such.

Many plant proteins are difficult to extract from plant materials and relatively difficult to retain in soluble form. Interest in sodium salicylate as a dispersing agent for proteins arises primarily from the fact that solutions have proved to be very effective in dispersing such relatively insoluble protein material as wheat gluten. It has a great advantage over acids or alkalis since it is usually used at neutral pH values, commonly at 6 0 to 6 8, and does not cause hydrolysis (Rose and Cook, 1935).

Cook and Alsberg (1931) and Cook and Rose (1935) showed that wheat gluten was "completely dispersed" in 8 per cent sodium salicylate. Rose and Cook (1935) reported that, using viscosity as the criterion, effective particle size of gluten was larger in sodium salicylate than in either acid or alkali solutions. Furthermore, the precipitate obtained on salting out the dispersed protein indicated that the salicylate altered the gluten less than did either acid or alkali. McCalla and Rose (1935) fractionated gluten dispersed in sodium salicylate, redispersed each of four fractions separately, and recombined the dispersions. When the recombined gluten was precipitated it possessed most of the characteristics of the original gluten.

Early sedimentation and diffusion studies with gluten fractions were carried out using alcohol as the dispersing agent (Krejci and Svedberg, 1935; Lamm and Polson, 1936). This meant that only the most soluble, or gliadin, fractions were studied, and it also meant that salt had to be added to overcome the charge effect. While these early investigations yielded interesting results, they were not very satisfactory to those workers interested in the study of whole gluten. Sodium salicylate solutions appeared to offer a good alternative dispersing agent for use in such determinations since the ionization of this salt itself was adequate to prevent the interference of the charge effect in sedimentation and diffusion experiments.

Even at this time, of course, it was recognized that gluten protein dispersed in sodium salicylate solutions was unlikely to exist in the same form as in the undispersed, washed gluten. Nevertheless, the fact that cohesive gluten showing properties of elasticity and extensibility could be recovered, indicated that the changes effected by the solvent were reversible.

Sedimentation and diffusion studies with gluten dispersed in salicylate yielded new and interesting data on this protein material (McCalla and Gralen, 1942). Evidence of heterogeneity and estimates of molecular weight range and of the tendency for aggregate formation, were obtained. Sodium salicylate appeared to be entirely satisfactory for such determinations.

More recently, electrophoretic studies have been carried out on a number of plant proteins using dispersions in sodium salicylate. The ionic strength of 8 per cent salicylate is, of course, very high for use in electrophoretic determinations. Nevertheless, electrophoretic analyses of gluten were successfully carried out and mobilities calculated (Colvin and McCalla, 1949). In later studies, less concentrated salicylate has been used.

Before starting the first extensive electrophoretic experiments with whole and fractionated gluten, Colvin and McCalla (1949) analysed egg white dispersed in standard phosphate buffer and in 0.1 molar sodium salicylate. The number and proportion of components were comparable in the two reagents.

The distribution of basic and dicarboxylic amino acids in various fractions of gluten suggested that electrophoretic analysis of gluten would show that the protein is heterogeneous in electrostatic properties. This suggestion was supported by the work of Schwert, Putnam, and Briggs (1944) who found that gliadin, a gluten fraction, was itself electrostatically heterogeneous; and by that of Scallet (1947) who found that zein from corn was decidedly lacking in homogeneity. Both studies employed aqueous alcohol as the dispersing agent.

It was therefore surprising to find that gluten dispersed in sodium salicylate acted as a single component electrostatically (Fig. 1)

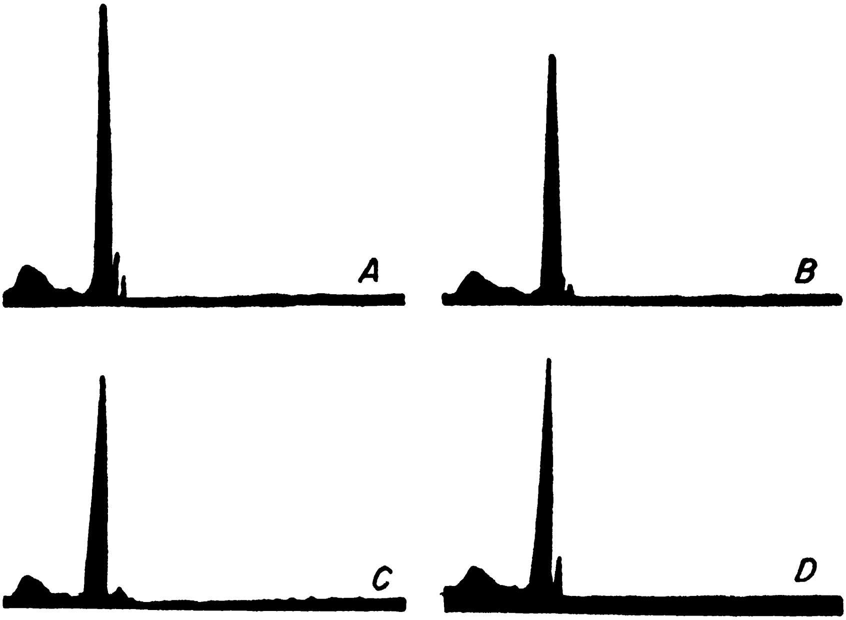


FIGURE 1.—Electrophoretic diagrams of wheat gluten dispersed in 8 per cent sodium salicylate at pH 6.8. *A* and *B*: whole gluten; *C* and *D*: molecularly dispersed fraction.

(Colvin and McCalla, 1949). Only one peak appeared in any diagram and the limited spreading of this peak did not indicate heterogeneity. This result was obtained over a wide pH range, although the lowest pH that can be used with this reagent is approximately 4.0. The salt loses its dispersing power below this point and precipitates as salicylic acid.

Another surprising result was that the mobilities of the protein decreased only slightly with a decrease in pH (Colvin and McCalla, 1949). This indicated that the isoelectric point must be well below pH 4, a result at variance with those obtained by other workers for gliadin and other comparable proteins. For example, Schwert, Putnam, and Briggs (1944) state that the isoelectric point of their fast gliadin fraction was at pH 7, while that of the slow fraction was at pH 5.

Just recently, Melnychyn (1951) has shown that gluten protein dispersed in formic acid–sodium formate buffer (0.1 M formic acid, 0.05 M sodium formate, pH 3.5) is electrostatically polydisperse (Fig. 2). The isoelectric point is well above this pH, but the buffer loses its dispersing power so rapidly as the pH is increased that no estimate

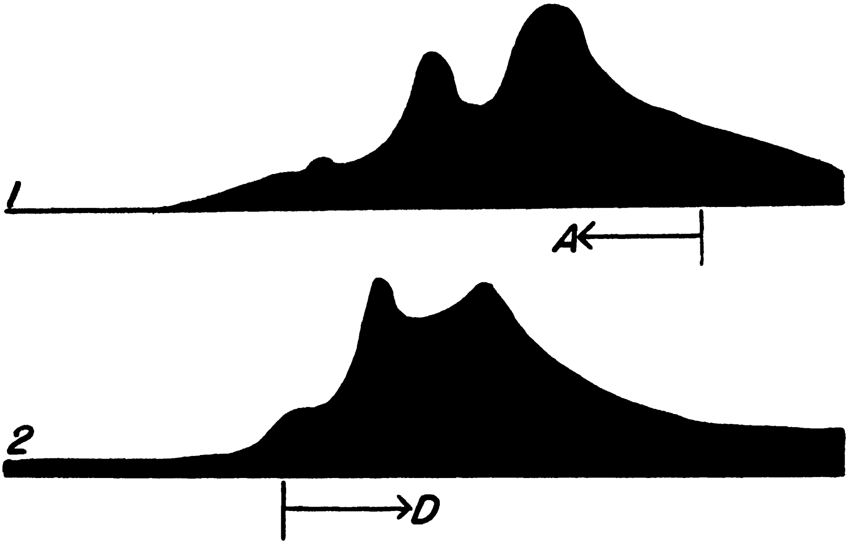


Figure 2—Electrophoretic diagrams of wheat gluten dispersed in formic acid–sodium formate buffer at pH 3 5 1: ascending boundary, 2: descending boundary.

of the isoelectric value can be made. It is certain, however, that it is in disagreement with the results obtained using sodium salicylate as the dispersing agent.

Similar results have been obtained for oat proteins. A large proportion of the protein in oat flour can be dispersed in 0.3 M sodium salicylate (Melnychyn, 1951) or in 0.1 M formic acid (Cunningham and Anderson, 1950; Melnychyn, 1951). Electrophoretic analyses have been carried out in both reagents. Examples of results are given in Figs. 3 and 4. Fig. 3 is for whole oat extract in 0.3 M sodium salicylate and Fig. 4 for a similar extract in formic acid. The protein in salicylate is much less polydisperse than that in formic acid. Furthermore, there is little or no spreading of the main component in salicylate and extensive spreading in formic acid.

One further example of the same type of result may be given with an entirely different type of protein. One of the main protein components extracted from the seeds of hubbard squash, *Cucurbita maxima*, is a crystallizable globulin. A second main component has not been identified but it has properties decidedly different from those of the cereal proteins already discussed. Nevertheless, Fuerst (1951) found that electrophoretic analysis of the whole protein extract in sodium salicylate fails to separate the main components as determined

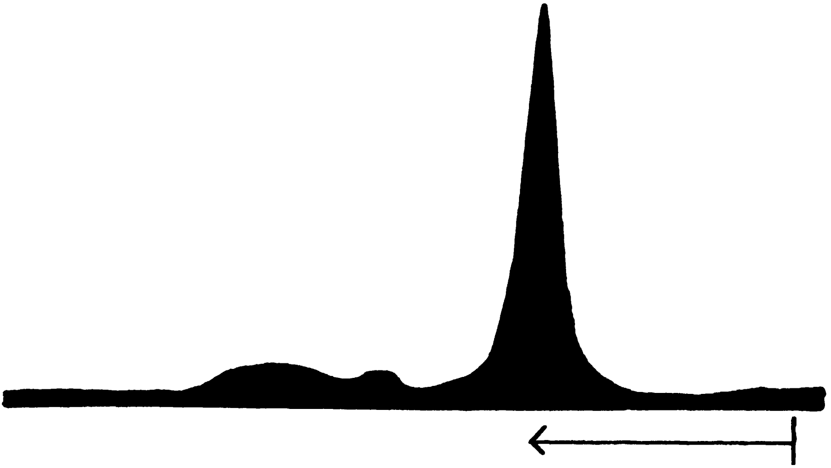


FIGURE 3.—Electrophoretic diagram of whole oat protein dispersed in 3 per cent sodium salicylate at pH 6.0

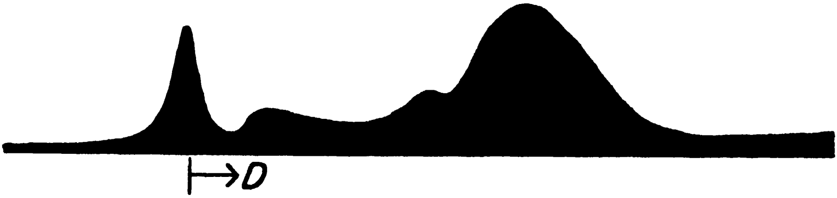


FIGURE 4.—Electrophoretic diagram of whole oat protein dispersed in formic acid–sodium formate buffer at pH 3.8

by analysis in several other buffers The dispersions in salicylate were always cloudy and satisfactory electrophoretic diagrams could not be photographically recorded. Only one sharp major component and possibly one very small minor component could be detected by observation of the moving boundary. In other dispersing agents, including phosphate and acetate buffers, two major components gradually separated and both spread rather rapidly indicating that neither was electrostatically homogeneous.

It therefore appears that only with egg white proteins has normal separation of components been effected by electrophoretic analysis of proteins dispersed in sodium salicylate. All the unfractionated plant proteins studied in the laboratory at the University of Alberta appear to be essentially electrostatically homogeneous in sodium salicylate, although all are shown to be heterogeneous in other dispersing agents. Since separation of components is one of the major reasons for electrophoretic analysis of proteins, salicylate has not proven to be a very satisfactory dispersion agent.

Investigations of complex formation between various proteins and many types of ions suggest that the effect of salicylate on electrostatic properties may be the result of such complex formation. Klotz (1950) has discussed this type of reaction, and the writer and his students have concluded that this offers the best explanation for the effects of salicylate.

It has been shown that essentially quantitative reaction takes place between reactive groups of the protein and some ions such as methyl orange (weakly bound) and dodecyl sulphate (strongly bound). It has also been shown that picrate ion and phthalate ion apparently react in this way with silk. Sookne and Harris (1939) describe this reaction as preferential adsorption, but in any case the reaction sometimes took place to the extent that the isoelectric point disappeared from the range studied. Colvin and McCalla (1949) found that molecularly dispersed gluten in sodium salicylate had a high negative "valence" of 34. Ion-protein complex formation could cause this high electronegativity, which in turn would explain the displacement of the isoelectric point so far to the acid side, and the flattening of the pH-mobility curve.

While much less work has been done with the other plant proteins, the same explanation of the results of electrophoretic analysis seems justified. If all reactive groups (of like sign) on the protein take up the salicylate ion, then the material being studied electrophoretically is the salicylate-protein complex. Components that were originally quite distinct, and that retain their distinction in other dispersing agents, lose it as soon as the distinctive reactive groups take up the salicylate ion. Thus two or more components may react with different amounts of salicylate, but the differences in the resulting complexes are insufficient to effect separation on subjection to electrophoresis.

The ions in the complex must be relatively loosely held since all the proteins studied can be recovered by precipitation. In the case of the oat protein, however, redispersion of precipitated protein in salicylate of the same concentration as was originally used cannot be effected (Melnychyn, 1951). This suggests that on disruption of the complex on precipitation the original structure is not recovered. The limited investigation does not justify any extended discussion of this result.

It is necessary, of course, to attempt to explain why egg white proteins do not give results comparable to those obtained with these plant proteins. The only explanation that fits the hypothesis advanced, is that egg white proteins either do not possess enough reactive groups to form an effective salicylate-protein complex, or that, if such groups are present, they do not react. Klotz (1950) discusses this question fully and concludes that the binding ability of a protein is not only dependent on the presence of reactive groups, but that the actual reaction of such groups may be determined by the nature of adjacent groups on the protein molecule. According to his results, egg albumin is intermediate in its binding ability, but no studies were reported using salicylate ion. It has been clearly demonstrated, however, that there are marked differences in the ion-binding abilities of proteins and that the response of a single protein differs towards different ions.

The main effect of the conclusions presented in this brief review is that no further electrophoretic analyses of plant proteins are being made in our laboratory with sodium salicylate as the dispersing agent. It seem quite certain that, with storage proteins at least, any form of dispersion means a change in basic properties. Differences in amino acid composition of components and fractions have been demonstrated, and these should, and in many instances do, result in differences in electrostatic properties of the dispersed protein. Since, with proteins in sodium salicylate, such differences disappear, and the isoelectric point is invariably shifted far to the acid side of the pH scale, salicylate must be considered as an unsatisfactory dispersing agent for use in electrophoretic studies.

REFERENCES

Anson, M. L. and Mirsky, A E. (1931). J. Gen. Physiol 14: 725-32.
Colvin, J. R. and McCalla, A. G. (1949). Can. J. Res. C27: 103-24.
Cook, W. H. and Alsberg, C. L. (1931). Can. J. Res. 5: 355-74.
Cook, W. H. and Rose, R. C. (1935). Can. J. Res. 12: 248-52.
Cunningham, D. K. and Anderson, J. A. (1950). Cereal Chem. 27: 344.
Fuerst, C. R. (1951). M.Sc. Thesis, University of Alberta.
Klotz, I. M. (1950). Cold Spring Harbor Symposia Quant. Biol. 14: 97-112.
Krejci, L. and Svedberg, T. (1935). J. Amer. Chem. Soc. 57: 946-51.
Lamm, O. and Polson, A. (1936). Biochem. J. 30: 528-41.
McCalla, A. G. and Gralen, N. (1942). Can. J. Res. 20: 130-59.
McCalla, A. G. and Rose, R. C. (1935). Can. J. Res. 12: 346-56.
Melnychyn, P. (1951). M.Sc. Thesis, University of Alberta.
Rose, R. C. and Cook, W. H. (1935). Can. J. Res. 12: 63-81.
Scallet, B. L. (1947). J. Amer. Chem. Soc. 69: 1602-8.
Schwert, G. W., Putnam, F. W., and Briggs, D. R. (1944). Arch. Biochem. 4: 371-87.
Sookne, A. W. and Harris, M. J. (1939). J. Res. Natl. Bur. Standards 23: 299-308.

TRANSACTIONS OF

THE ROYAL SOCIETY OF CANADA

SECTION V
BIOLOGICAL SCIENCES

THIRD SERIES—VOLUME XLVI—SECTION V
JUNE, 1952

OTTAWA
THE ROYAL SOCIETY OF CANADA
1952

TABLE OF CONTENTS

Levels of Analysis and Problems of Concept-Formation in Cytology. Presidential Address by C. Leonard Huskins, F.R.S C. 1

The Production of Life in the Bay of Fundy. Flavelle Medallist's Address by A. G. Huntsman, F.R.S.C. 15

Thyroid Function in Some Anadromous and Landlocked Teleosts. By William S. Hoar 39

"*Even-year*" *and* "*Odd-year*" *Pink Salmon Populations.* By Ferris Neave 55

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVI : SERIES III : JUNE, 1952
SECTION FIVE

PRESIDENTIAL ADDRESS

Levels of Analysis and Problems of Concept-Formation in Cytology

C. LEONARD HUSKINS, F.R.S.C.

"*Science is an interconnecting group of concepts and conceptual schemes arising out of experiment and observation, and fruitful of new experiments and observations.*"

IF one accepts this statement by J. B. Conant as a satisfactory brief definition of the type of activity that all of us here are engaged in, it may be of interest to inquire how some of the concepts of cytology, my special field, have developed in the past and are now developing. I think this inquiry will also reveal some of the changing ways of concept-formation that now characterize biology as a whole as it moves from its early descriptive stages to its present development in which it is in many phases linking up with chemistry and physics. Since our interests within biology and medicine are so diversified and every one of our fields is today very highly specialized, I shall take the liberty of sacrificing detail in favour of clarity through generalization, though I shall, of course, try to avoid distortion in the process.

We may roughly divide the history of cytology into three stages. We then find, in brief, that in the earliest period when the concepts were *thought* to come from direct observation of tissues and cells, they probably were influenced by extraneous ideas much more than was realized. Of course what we see is always determined by the previous experiences of our central nervous system; the difference is that we realize this more clearly than did most of our scientific forbears, partly because they thought their efforts were revealing Reality—with a capital R. In the second phase the concepts of cytology were greatly influenced by genetics; the relation was reciprocal and the hybrid science of cytogenetics developed rapidly. In the current stage of cytology the most exciting concepts, as in genetics, are embodying data and ideas from physics and chemistry; we are all striving as best we can to think in terms of function, that is really to become developmental physiologists. Of course any subdivision or classification of any science (or of anything) is to some extent arbitrary and at all stages there are, and must be, interactions between all sorts of ideas and all sorts of data. But it seems that to comprehend we must first subdivide and classify; the completeness or incompleteness of the synthesis (the view of the whole) that we make at any stage in the progress of our understanding, is in direct relation to our understanding of the parts, though it is always more than this.

Cytology began with the invention of the microscope. This followed the development of the telescope and I believe that many of the early errors of descriptive cytology arose from failure to recognize that at the terrestrial level these two instruments do two entirely different jobs. A spy glass, or a pair of field glasses, brings the distant but familiar into nearby focus; the microscope revealed a world new to our senses; the electron microscope is today revealing a still newer "submicroscopic" world which we have hitherto conceptualized chiefly from indirect evidence. To understand a new world we have perforce, as in all learning, to put the unfamiliar in terms of the familiar; Hooke's initial use of the word "cell" is an all too obvious example. How else can one explain the persistence for nearly a hundred years of the concept of amitosis or direct cell division other than as a transfer of the earlier observation that protozoa and bacteria "multiply by fission."

Let us examine some stages in the development and decline of this concept that cells divide merely by direct fission. The division of cells was probably first observed by Prevost and Dumas in 1824, but they did not realize its significance. Fourteen years later Schleiden and Schwann demonstrated the (almost) universality of cells. Their concepts of cellular reproduction, or rather production, provided, in general, that the new cells crystallized out of the nutritive substance or embryonic protoplasm. Considering how clearly cell division can be seen with even the simplest compound microscope, it seems that ideas derived either from crystallography or from creation mythologies (cf. the "Urschleim" concepts of the origin of life), or both, must have determined that, to us absurd, concept. Within a few years not only had it been demonstrated that cells arise by division but it had been shown that nuclear division precedes cell division. By 1848 Hofmeister had seen chromosomes. This later development may have been made possible by the development of the achromatic objective, which occurred between 1815 and 1830, but according to Hughes (1952), Fresnel in 1824 found that the contemporary achromatic microscope had no advantages over the earlier uncorrected objective at magnifications greater than 200x and Sachs records that as late as 1836 Meyen still preferred an English eighteenth-century instrument. Hooke, Leeuwenhoek, Grew, and Malpighi, we may recall, made their great discoveries late in the seventeenth century! Incidentally, if Schleiden and Schwann were wrongly influenced by primitive concepts, theirs is one of the few cases in cytology; it seems today that the ideas of Plato, once sophisticated, have been responsible for many more of the misconceptions modern biology has slowly discarded (or is still bedevilled by).

By 1855 cell division was recognized as the only method for cell reproduction and in that year Remak, studying blood cells in the chick embryo, pictured first a splitting of the nucleolus, next a splitting of the nucleus, then a division of the cytosome, then of the entire cell body, and finally of the cell membrane. He evidently had a passion for consistency! For nearly twenty years some such method of "direct nuclear division" was the only kind considered possible, but by 1876, after the invention of the oil-immersion objective, so-called "indirect division," i.e., mitosis, was considered equally important. In 1882 Flemming stated that mitosis was the almost universal process and that "direct division" or amitosis was very rare. But in the same year Strasburger claimed that both mitosis and amitosis were normal and that the mitosis occurring in higher forms was derived from the amitotic type which, he thought, still occurred in lower forms. In 1891 Flemming effectively disposed of Strasburger's argument by publication of *observations*. But more important for our analysis of concept-formation is the fact that the concept of amitosis as a mechanism for normal nuclear reproduction had been rendered virtually untenable in 1888 by Roux's critical analysis of the issue. He pointed out that mitosis is a complex process obviously requiring much energy as well as time; it must therefore have had a high selective value if it has replaced the much simpler mechanism of amitosis. The basis for this he thought obvious· mitosis provides a mechanism for qualitative as well as quantitative division, whereas amitosis would be effective only if the materials to be divided were undifferentiated granules. Amitosis survived in the text-books, however, for more than half a century longer; Kater reported as recently as 1940 that "disagreement is still rampant." Amitotic division probably does occur in degenerating nuclei and one can always see what look like direct divisions in some nuclei of normal tissues; but to accept these as evidence of amitosis as a normal process of nuclear division means that only a dozen years ago there were some biologists whose trust in the evidence of their eyes exceeded their trust in logical analysis of numerous diverse but very relevant data. Had they been consistent they would have believed the evidence of their eyes that the sun daily moves across the sky!

The concept of the continuous spireme, the idea that at the beginning of mitosis there is in the nucleus a continuous thread-like structure that breaks up and forms the chromosomes, has a very curious history. It apparently began with Flemming's earliest work—1879—in which he was more interested in the thread-like nature of the nuclear material than in the number of threads present. He did not pronounce a law of the continuous spireme; he merely assumed it as the apparently simpler concept in the absence of critical evidence that it was discontinuous. He observed that the threads were double very early in the prophase of mitosis and probably sensed that this provided the initial step in a mechanism for the production of two identical sets of longitudinally differentiated chromosomes to be separated into two nuclei, as explicitly formulated in 1883 by Roux. But Strasburger in 1884 was convinced that the thread was continuous, which led Rabl to say the following year: "To date all research on cell division is permeated by a concept which appears to be almost a hereditary sin. It is the supposition that in the beginning of the thread-ball formation a continuously wound thread travels through the entire nucleus. . . ." He reported that "although it is often possible to follow a single thread for some distance . . . with great patience one will regularly come to a point where . . . several threads fuse or the thread actually comes to an end." Then he asks: "Why should one think that the finding of one thread rather than several threads is something better, something more histological?" (We may add that the use and abuse of "Occam's Razor" is still a serious problem in cytology.) Three years later Strasburger abandoned the idea of the continuous spireme and attributed his earlier error to poor fixation. The argument, however, continued unabated for another thirty years. By that time it had got thoroughly confused with the problem of chromosome pairing in meiosis. Somewhere along the line the idea had arisen that in meiosis the chromosomes are initially associated only at their ends (possibly because the continuous spireme broke up only between each of the pairs of chromosomes instead of at every chromosome end) and that only later do they come to lie side by side. The chains of chromosomes found at metaphase in some species of Oenothera were thought to confirm this concept of end to end (telosynaptic) pairing. A much more complicated, and now well-established, explanation of the Oenothera case, for which the data were derived about equally from genetics and cytology, finally disposed of both telosynapsis and the continuous spireme concepts. How either of them came to be so widely accepted when all along there had been good descriptive evidence for the alternative view that the thread is discontinuous and that pairing is side by side, with the end to end arrangement secondary when it occurs at all, is hard to understand, especially since these alternative direct observations could be, and quite early were, logically correlated with developmental and genetic data and concepts. It sometimes seems that those cytologists who "refused to theorize," who stressed the value of direct visual evidence over that of indirect evidence from other fields, actually were subconsciously prejudiced against indirect evidence and therefore gave it a negative value. The situation has changed, and perhaps has gone to the other extreme, since about 1930!

That the thread-like structures of prophase form spirals which determine the sausage-like shape of the metaphase chromosomes of meiosis was seen by Baranetsky in 1880. Strasburger could not see the helix within the metaphase chromosome, and apparently only two cytologists, Vejdovsky and Bonnevie, did so (or had the nerve to say emphatically that they did) in the next forty-five years. This is not to minimize the greatness of Strasburger. Because the essential nature of scientific understanding is progressive and human nature is commonly conservative, the degree to which a scientist's ideas may be correlated with delayed formation of new concepts by his followers is one indication of his greatness. Since 1925 everybody has seen spiral structure; the concepts concerning its formation and the details of its form in mitosis and meiosis are another matter. Suffice to say here that the most complete unified theory has the largest microscopically-visible spiral depending for its formation on the existence of a smaller spiral which is at the limit of microscopic resolution, and which depends, in turn, on the spiral structure concept of protein molecules. It happens, however, that before this unified concept was formulated, analysis of the changes of direction in the spiral that can clearly be seen had effectively ruled out any such *direct* relationship between molecular spirals and the visible spirals. This was "the new cytology" of the 1930's: a logical theory was better than direct observations—observations that did not fit a logical theory were discredited. It was the earlier pattern in reverse!

From the formulation of deductively derived concepts that integrated cytological and genetic data in the 1930's came some enormous advances. But Schrader (1948) and Sturtevant (1951) have also indicated how unsound in detail were some of the hypotheses that have nevertheless done so much to unify thought and particularly to facilitate teaching in cytogenetics. I will here touch on only one other concept of this period, the one in which the basic error has been recognized by Darlington who propounded it and which has significance because its abandonment exemplifies what is, I think, undoubtedly a trend towards more complex concepts in both genetics and cytology. Some of these concepts are bound to be better than the earlier simpler ones; if some are erroneous they can hardly be more so than some of those derived from direct observations.

In 1903 Sutton had demonstrated clearly that the segregation of Mendelian genes parallels the segregation of the chromosomes during meiosis. The linear order of the genes on the chromosomes was gradually established and received final confirmation in 1935. This completed the proof that the four chromatids of a pair of conjugated meiotic chromosomes carry (or are) the four strings of Mendelian genes that are distributed to the four nuclei that are produced by the two divisions comprising meiosis. The corollary was that the Mendelian genes being the units of heredity, the chromatid must be, transversely, a unitary structure. But by that date many cytologists had reached the conclusion, from direct observations, especially of large chromosomes, that the chromatid is a two-, four-, or indefinitely multiple-stranded structure. The "precocity theory" which served so nicely both to embrace Mendelism and to "explain" the mechanism of meiosis itself rested on the idea that the chromatid was a single strand. To save the theory it was therefore necessary to claim that the observations of double or quadruple strands in a chromatid must be optical illusions or else that the chromatid, though morphologically a double structure, must be "physiologically" or "effectively" single. For twenty years the theory was "saved" by the first method. X-ray breakage data were predominantly used to *prove* that the observations of doubleness or quadrupleness must be erroneous. X-rays often break both chromatids of a chromosome, sometimes only one, and only relatively rarely cause the cytological or genetic effects that would be expected from half or quarter chromatid breaks. But actually such effects do occur and in up to 20 per cent of cases in some experiments! At first these were ignored; then they were explained away as delayed or secondary effects of the irradiation; finally, it was generally agreed that X-ray bombardment is too crude a method to settle the issue. Its use is analogous to the use of shrapnel when the need is for a precision rifle that will enable only one of two paired targets to be hit. Today the morphological multiplicity of strands in the chromatid is recognized by practically all cytologists and the concept of physiological singleness appears to be both unnecessary and at variance with some cytological evidence. Another obvious possible resolution of the conflict between the cytological and the genetic data seems to be gaining ground though it is not yet widely accepted: this is a change in the concept of the gene. At the level of ordinary Mendelian analysis the gene is a unit; at the level of the light microscope its carrier, the chromatid, is a double or quadruple structure, at the electron microscopic or the molecular level both may be multiple. That a multiple structure can be a unit in heredity is not too difficult to imagine; how it can be a unit in mutability (which is generally assumed but by no means proved) is a problem for the solution of which we have as yet no very promising suggestions (I have elsewhere, 1947, considered one). Perhaps we shall have to wait for further data on the structure of nucleoproteins. Certainly we shall have to await clarification of our concept of the gene; as I pointed out in our Symposium of two years ago (1950) this has long been more complex and less definite than is commonly recognized—Goldschmidt and McClintock are currently presenting crucial data that demand new concepts of the mutation process and of the nature of the gene. The "aperiodic crystal" as Schrödinger calls it, may help in forming new concepts of the gene and chromosome, though it seems hardly likely to be the all-embracing answer to the problem of "What is Life." Incidentally, *that* book reveals the need for more critical transfer of cytogenetic data by physicists who formulate biophysical concepts.

To conclude these remarks on the second period of cytology: whatever we may teach in elementary cytogenetics we shall not, at the research level, go back to over-simple reliance on direct observations. The electron microscope will provide direct evidence for or against some of our concepts of structure, but using it we shall have to remember that once again a new, unfamiliar world is opening up to our eyes and that it may be quite a long time before our interpretation of what we see in electron micrographs can be relied upon.

In our current, third phase it is becoming increasingly recognized that Mendel's great contribution to biology was not Mendelian ratios (they even misled in some important aspects) but the concept that heredity depends upon the transmission of differentiated material particles present in the nuclei of reproductive cells. As soon as this was fully established, old notions such as telogony, maternal impressions, and the inheritance of acquired characteristics became logically obsolete. They survive, particularly in Lysenko's brand of Soviet genetics, only because the basic principles of Mendelian inheritance either are not understood or are deliberately ignored (as was the double or multiple structure of the chromatid by most Western geneticists for at least a dozen years after it was sufficiently established). The particulate concept of matter in general goes back, of course, to Democritus (who was so hated by Plato that he wished all his books burnt). In biology it found definite formulation in Darwin's short-lived "provisional Hypothesis of Pangenesis" and in Mendel's now well-established laws. It is now linking cytology with chemistry and physics. We are lucky that we do not feel it necessary to consider the problem which it posed for scholastic logic. The dilemma to a scholastic philosopher is, as outlined by Herbert Spencer, that if matter can be divided into ever smaller particles then there must be an infinite number of particles and the human mind apparently cannot conceive infinity. On the other hand, if a particle cannot be further divided it cannot be represented, for every model of a particle must have three dimensions and therefore be capable of further division. We shall, of course, be happy if we can analyse living matter down to molecular "particles." The concept of levels of integration enables us to think in terms of diverse levels of unity. This philosophical digression is not, however, irrelevant; it is surprising how over-simplified the concepts of good descriptive cytologists can sometimes be; for instance, at a much higher level, particles that are at the limit of microscopic resolution have often been described as spherical, the fact that at that level particles of any shape will appear spherical, though well known, having apparently been overlooked. There are biologists who still think of protoplasm as composed of undifferentiated particles—not consciously, of course, but evidently unconsciously, since this thinking is inherent in some of the concepts they still accept. The logical paradox that Spencer cited will not bother us because we know (*a*) the limitations of our ordinary three-dimensional models at the molecular and atomic levels, and (*b*) that while indefinitely continued division is logically possible for undifferentiated matter there are definite limits to the subdivision of any structurally differentiated material Structure is fundamental in our general thinking; it is, as Frey-Wyssling makes clear, structure that is the fundamental feature of living matter—as we see the problem today. It is, to me, a startling fact that different structural arrangements and/or proportions of carbon, hydrogen, oxygen and nitrogen atoms are so largely responsible for the specificities of inconceivably vast numbers of proteins—specificities so significant they may determine life or death for an organism, as in antigen-antibody reactions. In the face of such facts as this, Aristotelian reasonings on form and matter become equivalent to a child's play with Tinker Toys and Hegelian or Marxian transformations of quantity into quality appear as the dawn of adolescent understanding.

Before going on to concept-forming in the current phase of cytology we might stop to consider the steps involved in the formation and breakdown of the law of constancy of chromosome numbers, since this process extends across all three stages. In brief it seems to have been somewhat like this: (1) the nucleus was found to be composed of chromosomes during the division cycle, but these were not observable during the resting stage; (2) they were therefore formed either from a network of chromatin or from a continuous spireme thread; (3) the number was found to be more or less constant; (4) genetic data seemed to require constancy of the genetic constitution of all cells produced by mitosis and the concept which related Mendelian genes to the chromosomes therefore demanded that the chromosome constitution be the same in all cells that are produced by mitosis; (5) genetic data demanded the maintenance of chromosome individuality during the resting stage when they could not be seen; (6) exceptional observations of deviant chromosome numbers were therefore attributed to error or the deviating numbers were assumed to be abnormal or the result of wounding or other pathological conditions, (7) once the chromosome theory of heredity was firmly established it was natural for everyone to begin looking for exceptions to it and the breakdown of the constancy concept began; (8) then it was recognized that both the cytological and the genetic methods of determining chromosome numbers were too crude—both could give an answer only for cells that were reproducing themselves; (9) indirect determination of chromosome number by the counting of heterochromatic chromosomes or parts of chromosomes that were observable in non-dividing cells revealed that high multiple numbers were often present, as had been predicted earlier from the occurrence of progressive size classes of nuclei; (10) stimulation to division, by means of auxins or wounding, of nuclei that ordinarily would not have divided again showed multiple chromosome numbers; (11) the concept of constancy of chromosome number is today recognized to be strictly limited, perhaps especially to germ cells and embryonic or meristematic tissues as Winkler, almost alone, had indicated in 1916, two years before the constancy concept was formulated as a "law." It may be added as a 12th point (developed later herein) that in the current third phase the chromosome content of nuclei is being estimated from the desoxyribose nucleic acid content as determined by photometric absorption techniques. Until very recently this could be done only in more or less homogeneous "resting" nuclei. A new technique developed independently by Ornstein at Columbia and by my colleague, Klaus Patau, now permits measurement of DNA in nuclei at any stage and even in individual chromosomes.

Leaving the chromosomes as such for the moment, but before passing on to the cytoplasm in general, we might list in passing the chief concepts of spindle formation. From direct observation there are concepts of fibres pulling or contracting, or of other fibres growing and pushing, of the whole spindle expanding, of streaming and diffusion, and so on. From colloid chemistry there are concepts involving viscosity and hydration. Electrostatic models have been made—the most comprehensive one to date seems far too simple. Consideration of hydrodynamic forces has led to concepts of which Schrader (1944) says some have "some potential value" but for which "some factual support is very badly needed." Interionic forces that produce tactoids are among the most recent ideas, but they have not yet led far. The biggest complication for any theory is that there appears to be chromosome autonomy in movement as well as chromosome submission to being moved by the spindle.

For the cytoplasm in general we have had many concepts. The original description of it by Dujardin in 1835 was: "a gelatinous substance, perfectly homogeneous, elastic, contractile, diaphanous, insoluble in water and without traces of organization"; from this we proceeded through the micellar concept of Nägeli, the reticulate, the alveolar, the emulsoid, the granular, the colloidal, and now back to a micellar concept that utilizes concepts of structural chemistry to explain the submicroscopically determined fibrils or micellæ. It was, I think, the "telescope error" that caused neglect of Nägeli's early micellar concept. "What holds the micelles together?" some cytologists asked, thinking in terms of bricks and mortar and neglecting to think of "forces" that come into play at submicroscopic dimensions.

This leads us to the next and final topic of this paper—the development of concepts of the physico-chemical composition of the chromosome. Unfortunately, the data that have to be encompassed are already so diversified and numerous that only the high lights of the issues involved can be touched upon. Chemical analyses of sperm heads long ago made it probable that the chromosomes are composed of nucleoproteins; biochemical concepts of the complexity and variety of proteins and the *relative* simplicity of nucleic acids early made it seem likely to most cytogeneticists that the specificity of the genes depended on their protein moiety. It has therefore been somewhat of a surprise to many when during the last few years it has been claimed: (1) that nucleic acids appear to be universally associated with self-reproducing bodies—chromosomes, plastids, mitochondria, centrioles, viruses—and (2) that the protein content of nuclei varies tremendously whereas there appears to be a high degree of constancy in total desoxyribose nucleic acid content. Sperm and pollen nuclei contain half the amount of DNA characteristic of the nuclei of embryonic or meristematic tissues while nuclei of many older tissues, where mitoses are rare or absent, show the same or some multiple of the amount found in nuclei of embryonic cells. Constancy of DNA per nucleus becomes meaningful if related to chromosome number; most of the evidence (though by no means all) indicates a degree of constancy or replication parallel to that of chromosome number in differentiated tissues. The relationship to the gene (when we have clarified and extended our concepts of it) may be much less simple! Elsewhere (1947) I have argued that for heuristic reasons we should distinguish, at least temporarily, between the gene of heredity and the gene of differentiation and development. This was also proposed by Darlington shortly afterwards. The DNA data seem to me to make this more than ever desirable; if, as seems probable, they give us a reliable method of estimating chromosome number in non-dividing as well as mitotic nuclei, they thereby give us direct evidence on the maintenance and distribution of the genome. That the gene of differentiation, or even the gene of "gene mutation," *sensu stricta*, is the same thing as the gene of simple Mendelian analyses (which is merely a segment of a chromatid) cannot at this time be asserted with any confidence.

Enzymatic digestion analyses of the giant salivary gland chromosomes of Diptera showed a protein framework and a concentration of proteins in the bands, which most cytogeneticists considered the loci of the genes. But when the first measurements of DNA in germ cells undergoing meiosis seemed to show a doubling at the stage when the paired chromosomes become visibly split into the four chromatids, which are the second level units, the gene strings, of Mendelian segregation, it seemed logical to assume that the essential component of a gene must be the nucleic acid. How then account for the extraordinary differentiation and specificity of the genes? The first, apparently easy, way out was, of course, the assumption that nucleic acids must be more complex and of much greater variety than hitherto supposed. Chargaff and others have since provided evidence for this, but so far there is no method of determining differences along the length of the chromosomes.

It is pleasant to record that there has not so far been any serious lining up of workers into two opposed schools on the protein vs. nucleic acid nature of the gene question (the word nucleoprotein itself forces recognition of interaction), nor even on the DNA constancy question, such as it seems probable would have occurred if the attitudes towards concept forming of any period up to about twenty-five years ago were prevailing today. All (or almost all) of us in cytology or genetics today realize that biological concepts have to be complex;

both genetics and cytology have become branches (or roots) of developmental physiology. We [illegible] forming to encompass, as far as our [illegible] data from genetics, from descriptive cytology using the apochromatic objective, and the newer phase contrast system, from electron micrographs and radioautographs, from the microspectroscope and various photometric devices for measuring "natural" or specifically stained structures, from use of the ultramicroscope and the polarization microscope, from the chemical analysis of isolated chromosomes and other constituents, from X-ray diffraction images and the effects of diverse radiations, in fact from almost every tool used in any branch of science that deals with the structure and/or transformation of matter. As an example of the use of tools taken from another branch of science: one of my colleagues, R. E. Duncan, is using a technique developed in chemotherapy for the investigation of the effects of certain chemical analogues of some of the important constituents of chromosomes.

The complexity and the obviously indirect nature of the data that must be used in current concept-formation in cytology in themselves ensure against over-simplification. In addition, all cytologists are today all too well aware that we must not place too much reliance on the direct evidence of our eyes and that all our mental models, such as that of the genes as beads on a string, or our physical models, such as the chromosomes which we construct of pipe-cleaners, plasticine, or rubber tubes, are useful for teaching the facts of segregation and crossing over, but may be grossly misleading if we take them too seriously as representations of what occurs within the living cell. We know that all evidence which we get from our senses is in a fundamental sense indirect and that the sense impressions we receive from the use of new tools and new techniques usually require translation, a process that may take considerable time.

Yet we also know that we can never neglect what we have grown accustomed to call the direct evidence of our eyes. Where we differ from the majority of the early cytologists is in our understanding of the processes of concept- or image-forming. They *knew* that man sees "not with but through the eye," as William Blake expressed it, but they did not always remember it. We are continuously aware that our eyes and the lens system of a microscope are alike components of a compound instrument which functions by transmitting electric impulses to the brain. And the image the brain makes from those impulses is influenced, or perhaps even determined, by the simpler images it has previously constructed from electric impulses received through the medium of any or all of our sense organs. Thus, through the eye we see chromomeres or bands across the chromosomes throughout their length and these correspond to the image geneticists have formed of gene alignment, which their brains derived from counts of the frequencies of different combinations of macroscopic characteristics. Our satisfaction when the cytological and genetic, the directly and indirectly formed pictures coincide must be similar to that of the structural chemist when X-ray diffraction patterns reveal spacings and arrangements in harmony with the models of atomic spacings he has constructed from his indirect evidence. The question of whether the chromomeres and bands are blobs of nucleoprotein or turns of a spiral is, like the problems of atomic structure, a new problem at a different level of integration. We know from such coincident evidence that we can trust our different senses to reveal consistent impressions, that our interpretations have reality, though they are not revelations of the Reality with a capital R that most of our scientific forbears thought it was the job of science to uncover. For our greater comprehension in this regard we are, I think, above all indebted to the physicists and physical chemists who in turn owe much to the mathematicians and to those analysts of the nature of science and scientific concepts who, within or outside of their own ranks, and whatever their academic labels, are, I believe, the most significant philosophers of the modern world. Through their efforts we are both learning the limitations of human knowledge and gaining the comprehension of, that is the capacity to handle, if not fully to understand, the atom of matter and the gene of life.

REFERENCES

HUGHES, ARTHUR (1952). The mitotic cycle: The cytoplasm and nucleus during interphase and mitosis. London: Butterworth's Scientific Publications.

HUSKINS, C. LEONARD (1947). Subdivision of the chromosomes and their multiplications in non-dividing tissues. Amer. Nat **81**: 401–34.

——— (1950). Genes Proc Roy. Soc Can., 3rd ser, **44**: 246.

SCHRADER, FRANZ (1944). Mitosis: The movements of chromosomes in cell division. New York: Columbia University Press.

——— (1948). Three quarter-centuries of cytology. Science **107**: 155–9.

STURTEVANT, A. H. (1951). The relation of genes and chromosomes. Chapter 6 of *Genetics in the 20th Century.* New York: Macmillan.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVI : SERIES III : JUNE, 1952
SECTION FIVE

FLAVELLE MEDALLIST'S ADDRESS

The Production of Life in the Bay of Fundy

A. G. HUNTSMAN, F.R.S.C.

OF all the activities of the universe, life is the most complex one of which we have knowledge. Its production requires a great variety of substances and somewhat special conditions. The chief substance required is water, in which such a great variety of chemical activities can readily take place. The chief condition is strong light, which provides energy for the activities. Hence, a thin layer of water, containing a great variety of substances in solution, lying between the air above with its various gaseous substances and the earth below with its various solid substances, and exposed to sunlight coming through the transparent air, seems an ideal location for the production of life. Shallow inlets from the ocean have such characters, and one of these is the Bay of Fundy. Since the ocean has too much of the dissolved substances or salts for many kinds of life, it is an advantage to have the ocean water diluted, as it is in this bay, by water discharged from land, through rivers—water that is relatively pure through having come via the air in distillation from the ocean under the heating effect of sunlight.

It is very difficult to determine at all accurately how much life is produced in any part of the ocean, and yet it is quite evident that there are great differences from place to place in the amounts produced. A square mile of deep water has more space, water, and solutes for production of life than has a square mile of shallow water, but it has no more sunlight, and no more contact with air and earth. Whatever factors may operate, the numbers of animals and the amount of organic matter found on the bottom indicate that the amount of life in the ocean not only does not increase with increasing depth, that is, room for life, but actually decreases and also that, apart from depth, it decreases with distance from shore. That fisheries are never very far from shore seems to reflect this factor in ocean productivity.

The standing crop, that is, the amount of life that can be removed at any time, is not dependable as a criterion of what is being produced, since there may be long-period accumulations of organisms, particularly in cold water. The amount that can be taken out year after year is the best indication of the productivity of any part of the ocean. In spite of their imperfections, fisheries statistics provide the best picture yet available of comparative productivity. The world production of fish is estimated (FAO, 1950) as being 25 million metric tons. Of this total, 95 per cent is taken in the northern hemisphere, which has much less water but more shore than the southern hemisphere. In the northern hemisphere, most of the fish are taken in the temperate zone. In the Americas, there are practically no fisheries in the arctic zone, and the tropic zone furnishes less than 5 per cent of the total production in the northern hemisphere. The Bay of Fundy lies in the middle of the temperate zone of high fish productivity. An understanding of the mechanism in this bay should show what conditions are required for high production of life in the ocean.

To solve the problem or to understand how life is produced in the sea, one must appreciate how very different that life is from what we know of life on land. In water, little if any effort is required for an animal to remain at any level above the earth. Except in shallow water, the earth below, that is, the sea bottom, is in darkness and cold, with the lifelessness of a winter night, because water gradually extinguishes any light that enters it. For a wakeful life with the activity that light and heat permit, animals must remain near the surface of the water, or must rise toward it during the day if they sink when inactive or asleep at night. With darkness below, with diffuse light above, and with much restricted vision in every direction, each sea animal lives in a very small and simple world. Unable to direct its course over the earth unl close to the bottom, it goes wherever the water circulates, with modifications that its movements to and from the surface and its swimming here or there horizontally may determine. Differing from plants in being able to seize its food, the animal best sees food that is above and silhouetted against the diffuse light from the sky; and when food is scarce it is only the wanderer that survives. For such life, circulation of the water in which it floats must determine where it will be found by man who lives on land.

The Problem

How the fisheries of the Bay of Fundy are produced became an important matter in the late 1920's, when it was proposed that the entrances to Passamaquoddy Bay, which is near the mouth of the Bay of Fundy on the border between the province of New Brunswick and the state of Maine, should be dammed to generate power. At those entrances is the focal point of perhaps the most concentrated fishery for locally grown fish that is known, the capture in weirs of herring, chiefly the small ones for canning as sardines. It was judged that the correlation of this focal point with peculiar hydrographic conditions was significant and that the dams would greatly alter those conditions (Huntsman, 1928). The United States and Canada set up a commission which conducted investigations from 1931 to 1933 to determine the extent of any adverse effects that the dams might be expected to have on the fishery.

The investigators carried out admirable hydrographic, phytoplanktonic, zooplanktonic, and fishery surveys. They were limited to less than two years of field work, which practically precluded more than a testing of any ideas in mind as to adverse effects. Unfortunately there was no background of knowledge of how the fishery is produced. Each specialist looked for a local favourable peculiarity that would be eliminated by the dams. The hydrographer found (Watson, 1936) that the exceptional mixing of water in the entrances to Passamaquoddy Bay, which would be stopped by the dams and which had been claimed as an important factor, forms a small and negligible part of the total mixing throughout the Bay of Fundy. The phytoplanktologists found (Gran and Braarud, 1935) that the mixing is detrimental to plant growth and that the sardine region which would be affected by the dams is quite low in production of the plants to start the food chain leading to fishes. The zooplanktologists found (Fish and Johnson, 1937) that the region is characterized by small volumes of the animals that serve as food for the herring and is relatively unproductive. The ichthyologist could not see (Graham, 1936) that there were necessarily any more herring in the region than elsewhere in the Bay of Fundy and neighbouring Gulf of Maine and considered that the very large quantities caught might merely represent favourable conditions for their capture. This last was an attempt to solve the problem of the greatness of the fishery; but only by casting doubt on the uniform conclusions, drawn from visual observation (herring live near the surface in summer) and from the results of various kinds of fishing, that the fish are exceptionally abundant in the region and that the focal point in their abundance is in the entrances to Passamaquoddy Bay.

The positive results of the investigations revealed no reason why there should be more fish taken there than elsewhere and some reasons why there should be less. This made more of a puzzle than ever of the fact that enormous quantities of fish continue to be taken year after year in the region centred at the entrances to Passamaquoddy Bay. Yet it seemed incontrovertible that very large quantities of herring are grown in the region and become in some places extremely fat in feeding upon planktonic animals that must come from the Gulf of Maine

(Huntsman, 1938). Transport of food for the herring and even of the herring themselves (Huntsman, 1934) was seen as a possible solution of part of the problem.

THE PICTURE

We are able to see a picture clearly when there are distinct contrasts. The Bay of Fundy presents very great contrasts in the quantities of fish produced in its various parts (Fig 1). The impossibility of making an accurate census of the fish in the sea prevents refutation of any claim that the differences in quantities of fish caught do not reflect differences in local abundance of fish. Nevertheless, critical consideration of facts warrants no other conclusion than that fishery results do reflect differences in fish abundance, although not perfectly. In Fig. 2, the waters of the Bay of Fundy have been somewhat arbitrarily divided among the various coastal districts for which fisheries statistics are reported. For each district, the number of pounds per acre is given as calculated from its area and from the amounts of fish landed at the local ports in 1941. Two facts stand out: The very high figure (147) for Charlotte County at the mouth of the bay on the west side, which is the Passama-

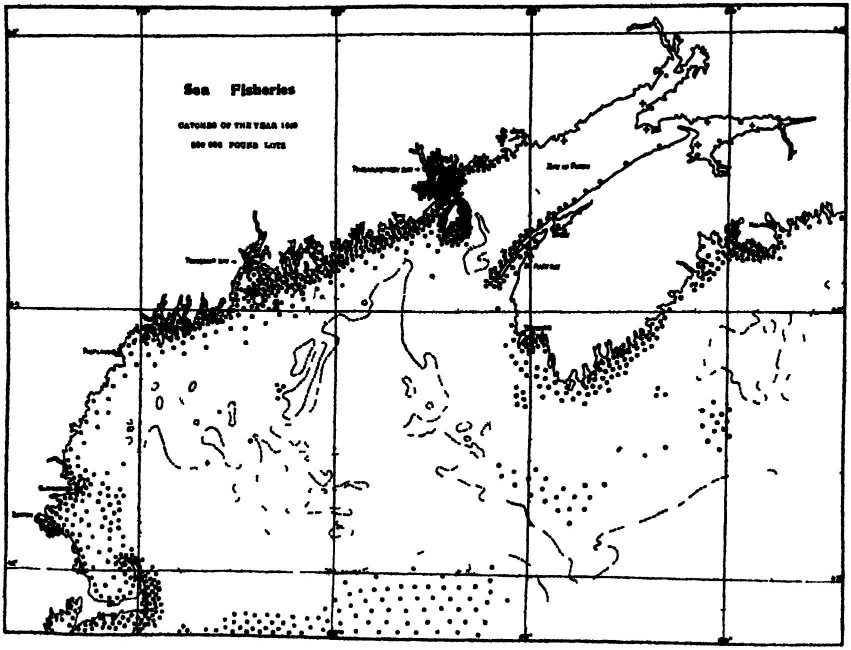


FIGURE 1 —Catches of fish from Cape Cod to Halifax in 1919, each dot representing 500,000 pounds Bay of Fundy at top on right Prepared by Dr A W H Needler.

quoddy region, and the very low figures (0.05 to 2.2) for the extensive inlets connected with the head of the bay. It will also be seen that the lowest figures (0.05 and 0.3) are at the head of the bay since there are somewhat higher ones (1.6 and 2.2) at the heads of the inlets. Another feature is that the figures for the New Brunswick shore of the bay are higher at the mouth and lower at the head than those on the Nova Scotian shore. Study has shown that these differences occur regularly. They constitute the picture, for which the causal background is to be elucidated.

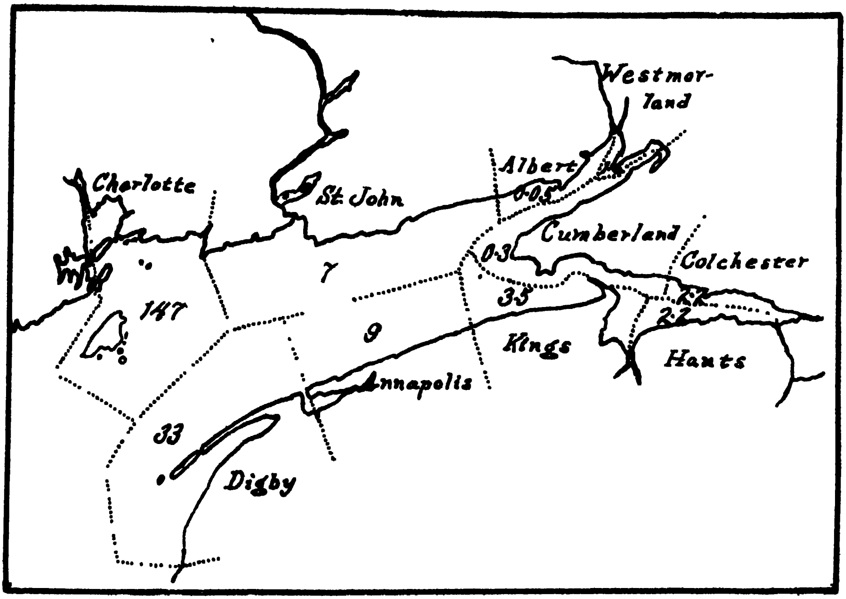


FIGURE 2.—Productivity of parts of the Bay of Fundy in pounds of fish per acre, based upon Fisheries Statistics of Canada, 1941.

The picture may be developed further where there are particularly favourable conditions. The higher production at the heads of the inlets is correlated with proximity to high discharge from rivers, and with heavy tidal mixing of this discharge with salt water. The St. John River of New Brunswick, which discharges into the Bay of Fundy about midway in its length, provides a very good test for the significance of this correlation, since it discharges a very large volume of water that comes from northern New Brunswick and Maine and from part of Quebec, and since its discharge is mixed abruptly and very thoroughly with twice as much salt water at a precise place, the Reversing Falls at the inner end of Saint John harbour. The relatively light mixture flows out and westward along the coast at the surface, and the salt water to reach the Reversing Falls flows inward at a lower level. However this may act, it has a strikingly precise relation to fish production

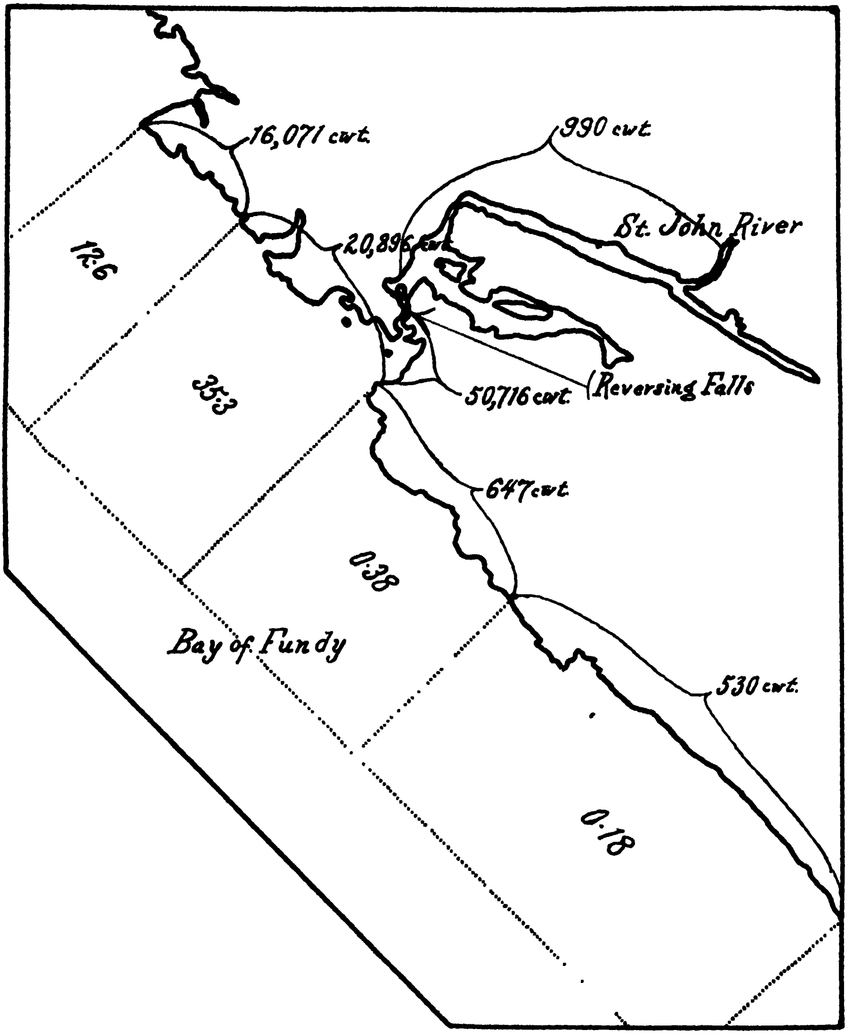


FIGURE 3.—Productivity of the waters of St. John County, New Brunswick, in pounds of fish per acre and in hundredweights of fish, based upon the fishery inspectors' reports for 1907, 41st annual report, Department of Marine and Fisheries.

as shown by the fisheries statistics. Separate figures for the landings in various parts of St. John County, which are necessary to reveal this picture, are available for the year 1907 (Fig. 3). Out of 8,886,000 pounds for the entire county, 5,071,600 pounds were for Saint John harbour, that is, for a small bit of water next to the Reversing Falls on the outside. The Kings County catch, which is for an extensive part of the slightly brackish estuary of the river inside the Reversing Falls, furnished only 99,000 pounds. For the coast outside the harbour, a fourteen-mile stretch westward along the course taken by the mixed water from the falls gave 2,089,600 pounds and a twelve-mile stretch further along this course gave 1,607,100 pounds. In most marked contrast, an eighteen-mile stretch eastward from the harbour gave only 64,700 pounds and a further stretch of thirty-three miles only 53,000 pounds. Whatever may be the explanation, the facts reveal a marked correlation between mixing of salt water with river water and high fishery yield.

In the earlier years for which fisheries statistics were collected, they were given in much greater detail. This has permitted more detailed development of the picture for the inner part of the bay. Two years were selected, 1876 and 1896, and these not only have provided the greater detail desired (Fig. 1), but they show differences from each other and from the picture for 1941 (Fig. 2). In the first place, more fish were taken in those earlier years, the poundage per acre for the whole inner part being 8.52 in 1876 and 8.6 in 1896, as compared with only 4.1 pounds per acre in 1941. Doubtless, with the fisheries of the inner part showing so little opportunity for development as compared with the outer part of the bay, the people turned to more lucrative employment. The fisheries in Charlotte County formed a decided contrast, proving to be well worth developing. They yielded 67.2 pounds per acre in 1876, 104.5 pounds in 1896, and 147 pounds in 1941. The greater details for 1876 and 1896 show that the higher yields at the heads of the inlets are related to river discharge, as is so clear for the Shubenacadie estuary, which discharges through Cobequid Bay into Minas Basin. The yields for it were 20.5 pounds per acre in 1876 and 27.5 pounds in 1986.

The greater yield for the Shubenacadie estuary in 1896 reflects a rather general situation in which there were greater differences in yields between the various districts in 1896. This is shown not only by the marked *decrease* in the yield in Cobequid Bay (from 16.2 and 3.25 pounds in 1876 to 3.4 and 0.6 pounds in 1896) as compared with the less marked *increase* in the yield in the connected Shubenacadie estuary, but also by the marked *decrease* in the yield on the north side of Minas Channel in Cumberland County (from 21.7 and 3.4 to 3 and 0.6) as compared with the less marked *increase* in the yield on the south side in Kings County (16.6 to 33.5). The facts have suggested, as presented above, that high yield is correlated with mixture of salt water with river water. Since river water is notoriously variable in amount, depending upon rainfall, it might be asked whether differences in yield are correlated with differences in river discharge.

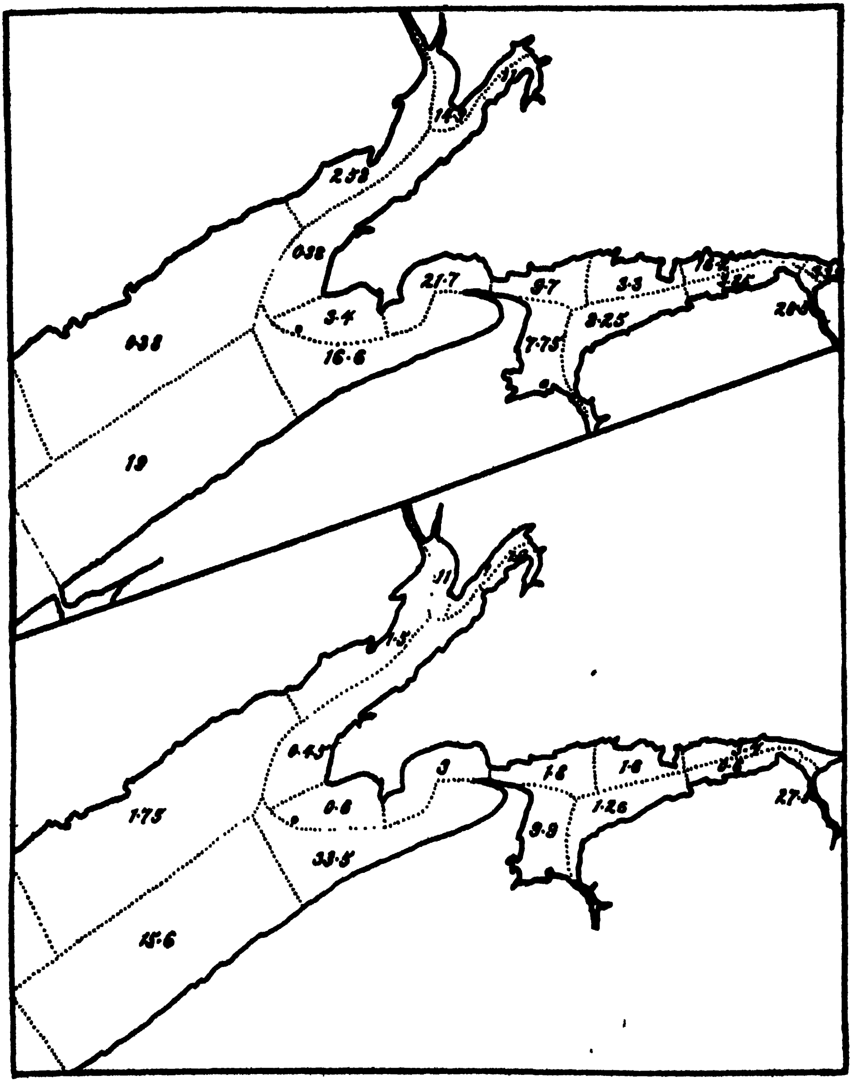


FIGURE 4.—Productivity of parts of the inner Bay of Fundy in pounds of fish per acre, based upon the fishery inspectors' reports for 1876 (top) in 9th annual report, and for 1896 (bottom) in 30th annual report of the Department of Marine and Fisheries.

While no records are available for river discharge in those years, there are records of rainfall for Truro at the head of Cobequid Bay and for Halifax near the head of the Shubenacadie River. The fisheries begin in April, are greatest in May, June, and July, and tend to end in August, as is shown by taking the monthly figures which are available for 1935. The amounts in hundredweights were reported as: March, 9; April, 130; May, 6,805; June, 7,287; July, 2,042; August, 453; September, 66; October, 0. The fisheries will be affected by the rainfall after the passing away of the spring freshets from the melting of snow and ice, which reach their peak in April. The mean rainfall in inches for Truro and Halifax for the months from May to August was: 1876, 4.74, 3.90, 2.97, 2.18; 1896, 2.00, 4.05, 7.46, 2.54. The rainfall decreased from month to month in 1876, but increased in 1896, and the total amount was one-sixth higher. With the spring freshet passing away and with plants using more and more water, decreasing rainfall has a marked effect on river flow. There is, therefore, a correlation between degree of concentration of fishery yield at places of marked mixing of river water with salt water and the amount of river water, as judged by rainfall. The Shubenacadie River water is mixed with the salt water in the estuary of the river. The water from all the rivers tributary to Minas Basin, after being mixed with salt water in the Basin and above it, is mixed with much salter water in Minas Channel, where the salinity of the mixture rises very considerably. In these places there was greater concentration of the yield in 1896 than in 1876 in correlation with increasing rather than decreasing rainfall during the season.

THE DYNAMIC SITUATION

It has been customary, as above, to consider water productivity in terms of pounds of fish per acre and to compare it with land productivity in pounds of beef per acre, as was done by Johnstone in *Conditions of Life in the Sea* (1908). This seems to imply, as was apparently assumed in the Passamaquoddy investigations, that fish depend upon food grown locally, just as cattle feed on the local grass. But is such a static conception as applicable to movable or moving water with floating plants and animals as it is to stationary land with rooted plants? The Passamaquoddy investigations clearly refuted this conception by showing that high local production of fish is associated with low rather than high local abundance of their planktonic food and with low rather than high local production of plants that form the primary food for the animals. However, it had already been clearly shown that this conception was inapplicable to the Passamaquoddy situation, which is a decidedly dynamic one. The herring had long been known to breed only outside the sardine region and to reach it as fry. The Bay of Fundy as a whole had been found (Huntsman, 1918) to have unusual physical conditions which render it unsuitable for successful breeding of fishes with pelagic eggs, such as cod, haddock, pollock, and hake. Such fisheries in the bay must, therefore, depend upon immigration of the fish. Also, these peculiar conditions had been seen to prevent successful reproduction of the lobster (Huntsman, 1923), and of certain floating invertebrates (Huntsman and Reid, 1921) which included the medusa *Aglantha*, the Amphipod *Parathemisto*, the Schizopod *Meganyctiphanes*, and the Chaetognath *Sagitta elegans*. The last of these was carefully investigated. The Schizopod *Meganyctiphanes* is an important food of the herring and is known to the fishermen as "shrimp." Investigation of the other main food of the herring, the "red feed" of the fishermen, which is largely the Copepod *Calanus finmarchicus*, had similarly shown (Wright, 1929) that this species "does not breed to any extent in the Bay of Fundy, but appears to enter that bay from the Gulf of Maine in the young state." This result for *Calanus* was confirmed (Fish, 1936a) and found to apply also to other forms of "red feed" namely *Pseudocalanus* (Fish, 1936b) and *Oithona* (Fish, 1936c).

Such evidence that the herring, which grow up in the Passamaquoddy region from the fry stage, depend for food upon animals from outside waters had made the static conception of fish production untenable, not only for Passamaquoddy Bay, but for the Bay of Fundy as a whole. Appreciation of the dynamic situation that exists is difficult for man, who from his position on land views and names the water in accordance with the basin in which it happens to be and tends to ignore the circulation which carries it and the floating plants and animals it contains from place to place over the earth. The situation is very complex, since some of the plants and the animals are attached to or remain associated with parts of the bottom, and since the locality or basin may determine to a considerable extent the character of both the water and the organisms therein. Nevertheless, the peculiarities in local fish production cannot be properly understood without taking account of the circulation that transports the organisms. Since this varies from season to season, the distribution of the organisms may be expected to exhibit great variety.

In no other part of the world is the dynamic situation for the ocean so clearly revealed as it is off our Atlantic coast. The coast extends from a degree or two south of the middle of the temperate zone northward into the Arctic zone. North of the middle, near the Grand Banks of Newfoundland, which jut out from the continent into the Atlantic, cold waters from the arctic zone and warm waters from the torrid zone meet more than a thousand miles from where they and the life they transport properly belong. The Labrador Current carries in summer icebergs from arctic Greenland, in winter field ice with Greenland seals and other arctic mammals, and at all times varied arctic life. The so-called Gulf Stream carries Sargasso weed with its associated animals from tropic seas and at all times contains varied tropic life.

The arctic water works southwestward along our coast from Newfoundland in great eddies, and masses of the tropic water are from time to time cut off in the oceanic whirls south of Newfoundland and enter these coastal eddies. The mixed water in these eddies carries both arctic and tropic forms of life. Each form goes only as far as it is permitted to reproduce successfully or merely survive, by virtue of (1) the proportions of the contrasted constituents, (2) the rate at which the mixture flows, and (3) the strength of the local conditions including admixture of water from the land, that alter the character of the mixture. Although over five hundred miles along the course of this mixed water, the Bay of Fundy receives from time to time considerable numbers of both arctic and tropic organisms Barrels of capelin (*Mallotus*), an arctic fish, have sometimes been taken in it, although this fish is not known to breed successfully south of Newfoundland. The arctic white whale, or beluga (*Delphinapterus leucas*), of which a colony lives in the icy cold waters of the St. Lawrence estuary at the mouth of the Saguenay River, has been seen in Passamaquoddy Bay. In 1951, an immense sluggish ocean sunfish, *Mola mola*, from the Gulf Stream was taken on the Fundy coast west of Saint John, and several of the tropic jelly-fish *Physalia*, the Portuguese man-of-war, were found at one of the entrances to Passamaquoddy Bay. Also, salps (*Salpa fusiformis*), which are tropic floating tunicates, entered that bay in swarms to die and be cast up on the beaches.

GROWTH OF PLANTS

Plants start the life cycle, since they alone have the ability to store energy from the sun for life activities, by building organic substances when in strong light. Production of life in the Bay of Fundy depends upon growth of plants there or elsewhere. Light is rather rapidly extinguished on entering water, and plants can grow only as far down in the ocean as sufficient light penetrates. This limits the attached forms to quite shallow water along shore and growth of the floating forms to a rather thin surface layer. The depth at which the amount of photosynthesis, that is, the building of organic substances, by plants just balances their respiration, that is, their use of these substances, is known as the compensation point, and their growth is possible only at a higher level. This depth is over 100 metres in the clear and rather barren waters of the Sargasso Sea in mid-ocean and tends to be low and variable in coastal waters, being as little as 2 metres deep at times in the Firth of Clyde (Clarke, 1939). Gran and Braarud (1935) found that in the outer Bay of Fundy heavy tidal action and discharge of detritus from rivers make its waters very turbid so that sunlight does not penetrate very far. An experiment indicated that in Passamaquoddy Bay the compensation point is no deeper than 10 metres. Graham (1936) measured the transparency of the waters of the outer Bay of Fundy and neighbouring Gulf of Maine with the Secchi disc, getting values from 4.0 to 14.0 metres. The lowest were in Passamaquoddy Bay and in the St. John outflow, and the highest near Cape Sable at the mouth of the Gulf of Maine. In 1951, Mr. L. C. Dickie made readings with the Secchi disc in the outer part of Minas Basin, in Minas Channel, and thence along the Nova Scotian shore to Digby Gut. Values in the basin ranged from 1.25 off Kingsport to 4.25 metres near the north shore, in the channel from 3.00 to 3.75 metres, and along the shore of the bay from 5.75 to 10.25. As is clearly evident, the inner part of the Bay with its heavier tides and shallower water is more turbid than the outer part.

It is not merely a matter of how deep in the water there is sufficient light for plant growth. Since the floating plants (diatoms) consume as high as about one-fifth of the amount of substance they build up under the very best light, they will not grow if, through their being carried up and down by the turbulence of the water, the total light they receive is as low as one-fifth of what they would receive under continuous light of optimum intensity. In the outer part of the Bay of Fundy, the phytoplankton is poor and the poorest places are those with most turbulence, which show poor growth even in spring (Gran and Braarud, 1936). It may be safely predicted that the inner part of the bay with its more turbid water and greater turbulence is even poorer in phytoplankton.

If the Bay of Fundy is so poor for plant growth, any abundance of animals in it must enter from the Gulf of Maine. The latter shows very different conditions. There is a rich phytoplankton everywhere in April and May and this continues throughout the summer along the coast where there is turbulence. The explanation of this is that, where turbulence fails, the phytoplankton exhausts the supply of certain salts (phosphates and nitrates). Turbulence maintains the supply by bringing unexhausted, rich water from the depths and solid particles holding these salts from the bottom. Also, the light, mixed water that issues in large volume from the Bay of Fundy and moves along the coast of

Maine is rich through not having had its supply of these nutrient salts exhausted by plant growth (Gran and Braarud, 1935). The great turbulence throughout the Bay of Fundy not only largely prevents plant growth in that bay, but also provides a steady supply of rich water for plant growth to the neighbouring part of the Gulf of Maine, water that is relatively light because it contains fresh water from the rivers, chiefly the St. John, that discharge into the Bay. The bay and the gulf may be said to co-operate in producing steadily a very large amount of phytoplankton in the gulf near the mouth of the bay. The animals that take advantage of this food are somewhat in a position to be carried into the bay if at the right depth for the return current of deep salt water.

TRANSPORT OF ANIMALS

The principal animals in the Gulf of Maine to feed upon the phytoplankton are Crustacea, the Copepods, and the Schizopods. *Calanus finmarchicus* and *Meganyctiphanes norvegicus* are the outstanding representatives of these two groups and both descend to deep water in sunlight. The order in the diurnal, vertical migrations of these and other zooplanktonts, including fishes, is as yet only partly elucidated. Since the currents in the Bay of Fundy and the Gulf of Maine vary considerably with depth, the depth at which these animals are swimming at any time determines where they will be carried. The complexities of their vertical movements and of the currents are so great that only major facts in the occurrence of the animals can with any confidence be attributed to specific transport, in accordance with our present knowledge of the water circulation and of the main vertical distribution of these animals.

Three types of circulation come prominently into consideration. Oscillatory movements of the water, such as the flood and ebb of the tide, bring about circulation through the action on the moving water of the rotation of the earth. This so-called Coriolis force diverts movements to the right in the northern hemisphere. Consequently, the water in a basin, such as the Bay of Fundy, circulates in a counter-clockwise fashion (Fig. 5). The mixing of light river water with heavy sea water in a channel (Fig. 6, bottom), as at the Reversing Falls of the St. John estuary, sets up hydrodynamic forces which drive the mixture oceanward at the surface and the sea water riverward at a lower level, and this has been studied for the St. John outflow (Hachey, 1935). When light water accumulates at the surface (Fig. 6, top), as in the centre of Passamaquoddy Bay from the outflow of the Digdeguash and Magaguadavic estuaries, tidal mixing along an adjacent rough shore produces a triple circulation, both surface and deep water moving to the place of mixing and the mixture moving from the place at an intermediate level (Watson, 1936). The dependable tidal currents of the Bay of Fundy act with the discharge of river water to ensure (1) a horizontal differential in movement that gives counter-clockwise circulation around broad basins, (2) a vertical differential in movement that is related on the seaward side to channel mixing of river water with sea water and that consists of movement from the mixing place at the surface and movement toward it at a lower level, and (3) a triple vertical differential in movement that is related to shore mixing of stratified water and that consists of onshore movements both at the surface and at some depth and of offshore movement at an intermediate level.

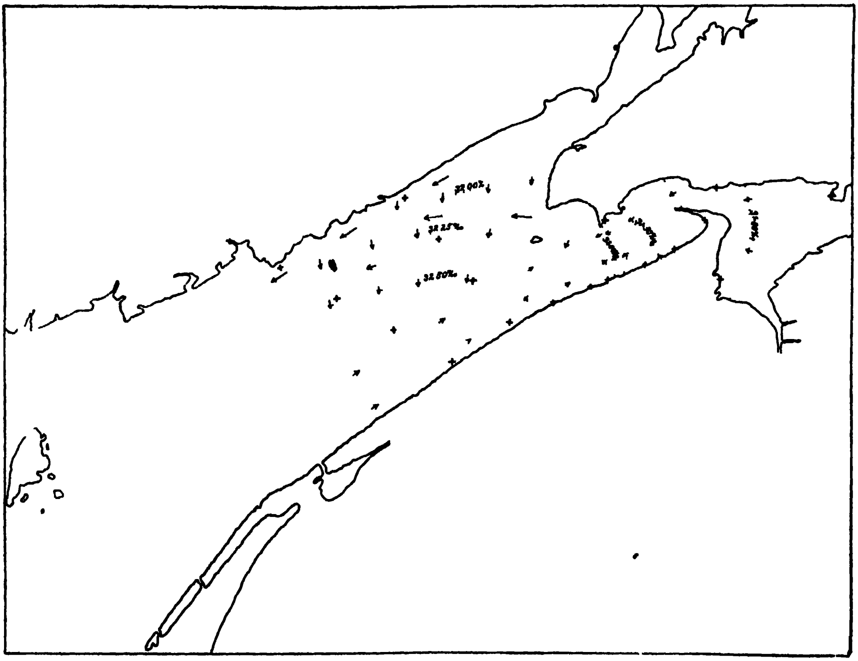


FIGURE 5.—Isohalines to show salinity of water in the inner part of the Bay of Fundy and the outer part of Minas Basin, based upon Watson's (1936) data for September 8, 1932, at eight stations in the bay, and upon data obtained in 1947 at ten shore stations related to Minas Channel All stations are indicated by crosses. Arrows represent circulation of the water as deduced from the above.

Three types of animals that differ in the vertical distribution which affects their transport in the water may be distinguished. *Calanus* and *Meganyctiphanes*, which on the whole keep far down from the surface, are in position for transport shoreward or riverward where such movements are sufficiently deep. The Copepods *Acartia* and *Eurytemora*, which on the whole keep near the surface, are in position for transport shoreward from the middle of basins containing stratified water. The herring, which keep near the surface when small and go deeper with

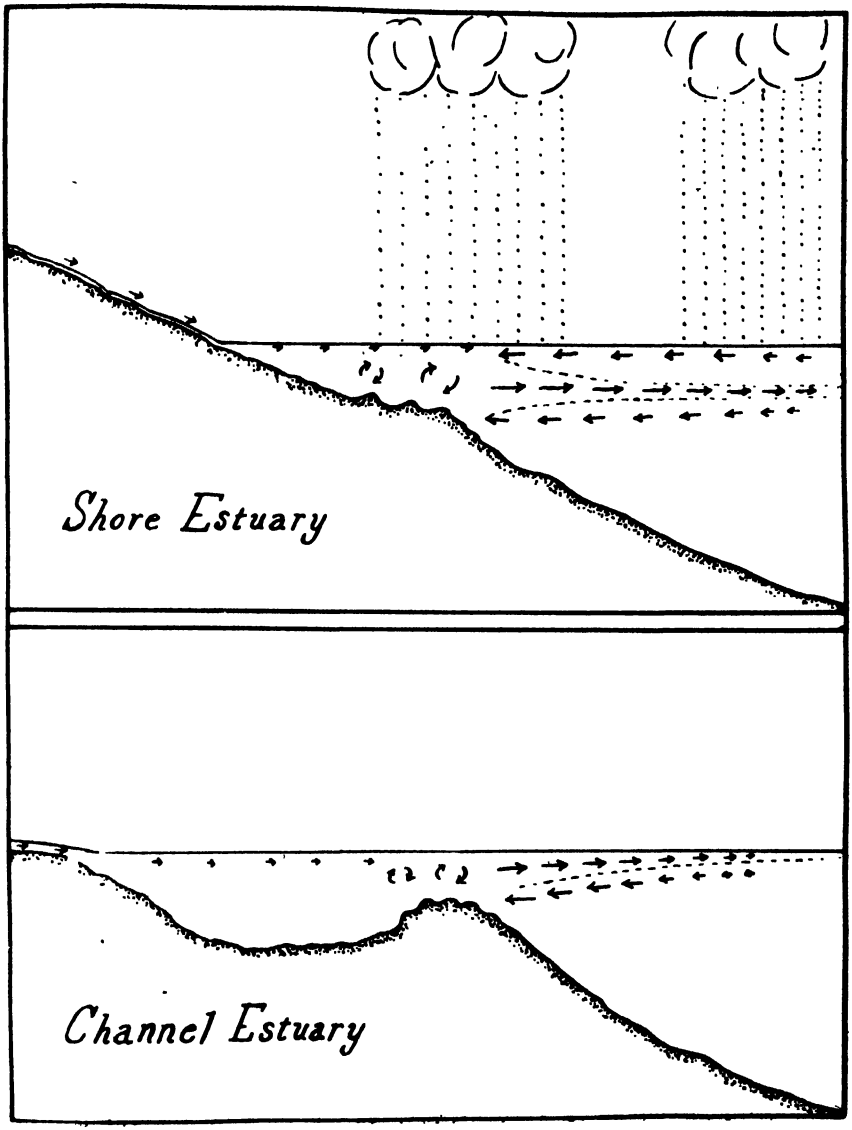


FIGURE 6.—Schematic representations of circulation engendered by shore mixing (top) and channel mixing (bottom) of light and heavy water.

increase in size, but are mainly not far from the surface except at low temperatures, are well fitted to show variations in transport with size and with season.

SOLUTION OF THE PROBLEM

At this stage in fisheries research, any solution must be considered as tentative and to be tested by much investigation. The knowledge outlined above does seem, however, to permit presentation of concrete accounts of how life is being produced in relation to the Bay of Fundy that serve to explain the great differences in productivity which the fisheries reveal.

High productivity in the outer part of the bay is clearly dependent upon indraught of food animals, if not of the fish, from the Gulf of Maine. The herring, which are responsible for the exceptionally great productivity of the Passamaquoddy sardine region, are present there throughout the year. When the adults were very numerous in the middle of the last century before the sardine industry developed, they were taken in the summer in great quantities on the outer side of Grand Manan Island, where they spawned. The spent fish could be taken during winter in such great numbers inland, even toward the head of Passamaquoddy Bay, that a big fishery for them was developed. The young enter that bay in the late larval or "eye-ball" stage, and the next stage, the britt, which is too small for canning, is concentrated there. The mechanism for this concentration is seen to be that, as a result of their habit of keeping to the surface, they are carried from the stratified water of the outer Bay of Fundy to the mixing places just outside and in the entrances to Passamaquoddy Bay. The Coriolis force ensures slow circulation of water into that bay through Letite Passage which is on the right going inward. Inside the bay, extensive mixing of the stratified water near the shore, from the middle of the west, or inner, side to the head takes such surface forms thitherward and thus holds and concentrates them in the bay. As the herring get larger, they go deeper in the water and tend to be carried from the mixing places to the centre of the bay and thence, in the outward movement, from the bay, which from the action of the Coriolis force is through Head Harbour Passage on the right going out. The larger the herring get, the farther out are they distributed on the whole (Fig. 7), until as adults they are almost entirely outside Grand Manan during the summer.

The very great quantities of these herring, which are brought to the region as larvae and which are largely held in it throughout their lives, must have food. Their growth shows that food is brought to them in ample quantity. That it is brought to them in more than ample quantity in some places and at some times is shown by the extraordinary fatness of those taken from Head Harbour Passage, or Quoddy River. Before canning of the young began, "Quoddy River herring" of unexampled fatness were fished in that passage, these being at the stage before spawning when herring are fattest. On occasion, the deep-water Schizopods or "shrimp" that must have come from the Gulf of Maine are cast up on the Campobello shore of the passage in such great quantities as to be carted away for manure. Also, I have been in a weir on that shore when it contained herring that after many days confinement were still unsaleable from being full of "red feed," and I found *Calanus* so abundant as to make the water feel gritty. The mechanism that makes this food available to the herring that are near the surface consists first of the deep return current that brings these deep-water forms from the Gulf of Maine to the deep water just outside the passage. Next is the working out through the passage of the mixed water from

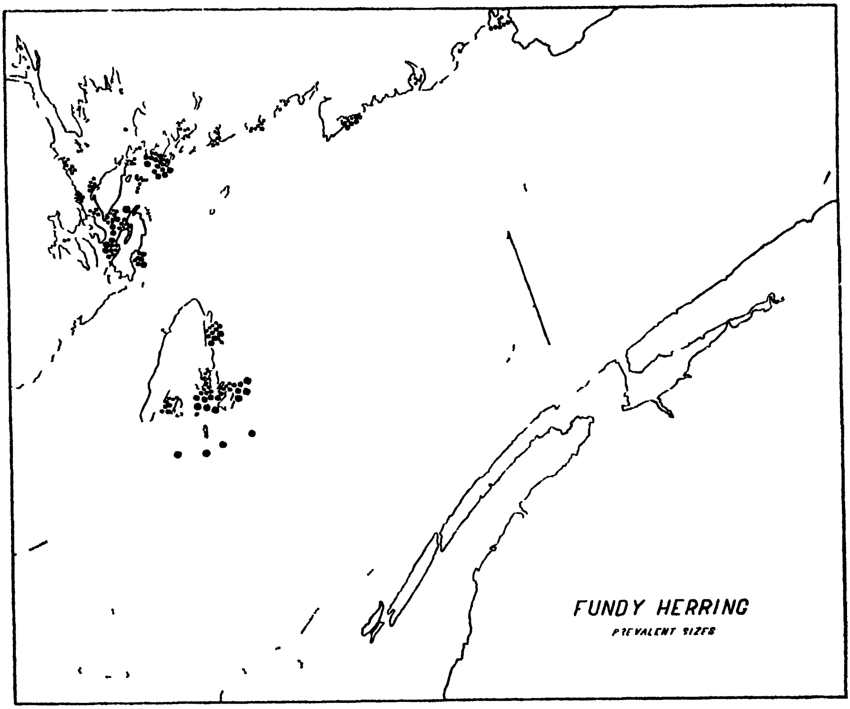


FIGURE 7.—Schematic representations of differences from place to place from Saint John to Passamaquoddy Bay and Grand Manan Island in the prevalent sizes of the herring taken, as reported in 1933 by four different men who transport fish to the factories of the region.

discharge of a heavy freshet into Passamaquoddy Bay. As found in 1933 and again in 1951, the strong reflux that this engenders brings the deep-water forms into the Passage in large quantity. There, they are brought to the surface in the tidal boils and carried by the tidal flow into the adjacent shallow inlets of Harbour de Loutre and Cobscook Bay, where they are rather steadily available to the fish. Their tendency to keep deep down in the water unless carried upward ensures that they are not removed from the passage in the superficial outflow, but remain to serve as food for fishes and also, at the very surface, for birds.

The small herring or britt that are concentrated in Passamaquoddy Bay grow too rapidly to become very fat. Their food is not provided through Head Harbour Passage, which is fully occupied by the outflow from the bay. The Coriolis force determines inflow through Letite Passage, which, although shallow, is so short that quite salt water enters during the latter part of the flood tide. This water is a mixture of surface and deep water from outside and thus contains both surface forms, such as *Acartia*, and deep forms, such as *Calanus*. Inside the passage, the water is rather deep and little turbulent. The deep forms descend and fail to be carried back out with the ebb tide and accumulate in the bottom water of the bay. The surface forms are carried back out to some extent, but, on the whole, work inward. Like the small herring, they are held in the bay because they are carried to the mixing places on the west shore and at the head. The deep forms are also carried to the mixing places, where both kinds become concentrated with the britt, thus permitting the latter to feed upon them. In 1951 this concentration of the britt and of the surface and deep Copepods was so favourable for the feeding of the britt that in August the surface of the bay was thickly peppered with their excreta and when they were large enough in the fall for canning, more than ten million pounds were taken by the weirs near the mixing places.

The small herring are not only concentrated with suitable food animals, but they are segregated from the larger herring which would compete with them for food. In addition, they are kept from their enemies. The mackerel (Fig. 8), for example, and also the dogfish are carried into the bay along the Nova Scotian coast, where they occur in fairly large numbers, but few of them reach the Passamaquoddy region.

Head Harbour Passage and the inner part of Passamaquoddy Bay provide the most striking illustrations of the process by which the large quantities of herring are produced that are taken in the Passamaquoddy region from Saint John to the mouth of the Bay of Fundy. The very heavy discharge from the St. John River, which is mixed with an immense amount of sea water as it passes out through this region, causes the circulation that brings the animals in from the Gulf of Maine. Through this circulation, the region is able to draw upon the Gulf of Maine as well as upon the Bay of Fundy for its supplies of larval herring and of herring food.

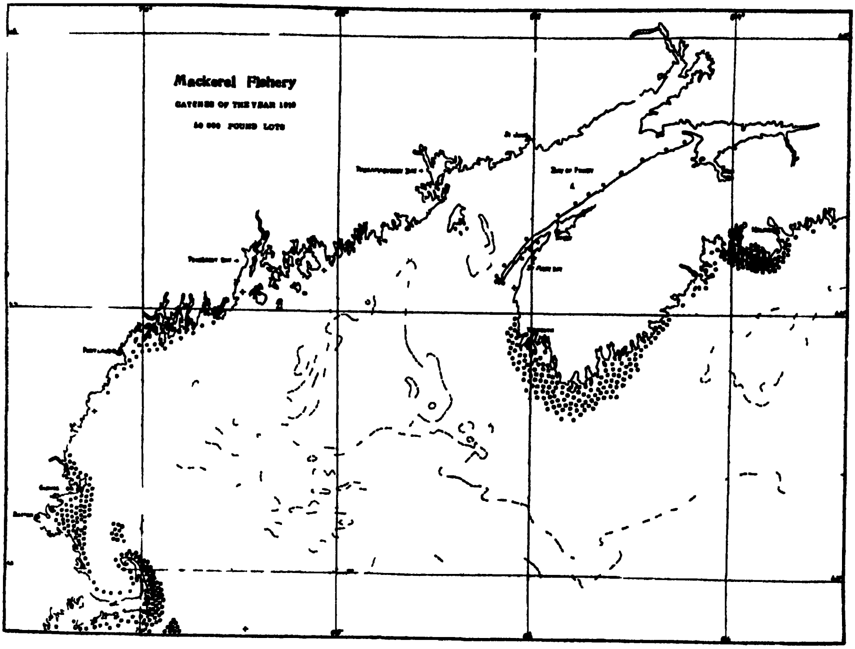


FIGURE 8.—Catches of mackerel from Cape Cod to Halifax in 1919, each dot representing 50,000 pounds. Bay of Fundy at top on right. Prepared by Dr A. W. H. Needler.

The concentration of fish near and inside Saint John harbour as far as the Reversing Falls is a different, but cognate phenomenon. While food animals for the fish are brought riverward in the return current, even as far as the harbour, this current is in part so near the surface where the outflow from the harbour spreads out over the bay (Fig. 9) that near-surface fishes such as the wandering adult salmon are carried riverward and concentrated in and near the harbour, where their capture is then particularly easy and profitable.

The inner part of the Bay of Fundy has such turbulent water that the deep forms such as *Calanus* that enter the outer part from the Gulf of Maine are carried but slowly into it. They are almost as often near the surface in the outward moving water as near the bottom in the inward moving water. Owing to the Coriolis force, there is a general inward movement along the Nova Scotian shore so that planktonic surveys show more fish food from the Gulf of Maine along this shore than along the other, just as the fishery results show more fish. The inward movement ends rather abruptly at the mouth of Minas Channel, where there is no longer scope for the Coriolis force to operate. The very great turbulence in this Channel, by moving the animals up and down, prevents forms that ordinarily are deep in the water from being carried inward by the inflow near the bottom, and forms that ordinarily are near the surface from being carried outward by the outflow near the surface. Floating material, such as branches of trees and fishing buoys, that may be brought to the mouth of the channel in the inward movement along the Nova Scotian shore of the bay are prevented by the surface outflow from penetrating the channel, and accumulate at the mouth in a dense mass that is known locally as the "cedar swamp." Drift bottles similarly reach the mouth of the channel and rarely penetrate it for any

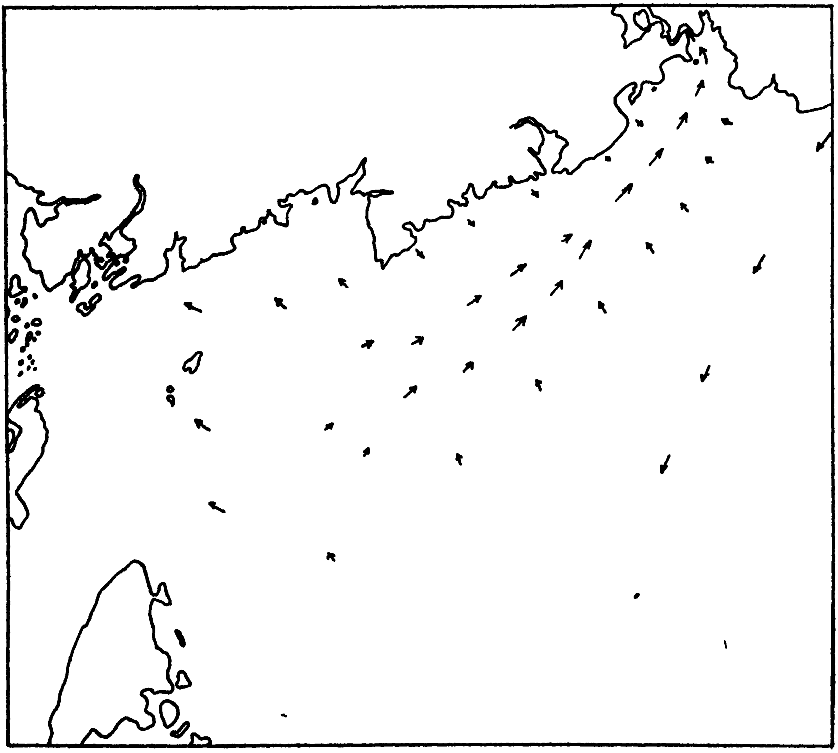


FIGURE 9.—Schematic representation of sub-surface circulation in the St. John outflow from Saint John to Grand Manan Island, as deduced from salinities.

distance. Fish that keep deep in the water in spite of the turbulence are carried in, so that some cod, haddock, hake, and halibut are taken inside Minas Basin. Lighter water on the New Brunswick side of the inner bay produces surface movement across the bay from New Brunswick to Nova Scotia. This enhances the difference between the two sides of the bay, making the New Brunswick fishery much poorer than that of Nova Scotia.

As judged from the plankton, Minas Channel is the most barren part of these waters in fish food (E. Jermolajew, unpublished). Crustacea from the Gulf of Maine just fail to reach it and, although not yet investigated, local growth of plants would seem to be at a minimum in spite of the richness of the water in nutrient salts. That phytoplankton in the inner Bay of Fundy is inadequate to provide food for the growth of microcrustacea which serve as food for fish is shown by the condition of the few *Calanus* that are carried far into the bay. They have been found to be nearly devoid of the oil that is so conspicuous as a very large globule in a well-fed individual. Those that enter the Passamaquoddy region from the gulf have more oil in them than the herring can digest, as is indicated by the fact that the masses of herring that have fed on *Calanus* can be readily located by the oil "slick" made by their excreta on the surface of the water.

That the amount of fish increases from Minas Basin to Cobequid Bay and to the Shubenacadie estuary requires explanation. If low production of phytoplankton in the inner bay and in Minas Channel is due to the turbulence preventing the floating plants from remaining long enough sufficiently near the surface to receive the amount of light required for their growth, the amount of growth should increase as the depth decreases, since the shallower the water the less far will they be taken from the surface by the turbulence. This matter has still to be investigated, but study of the zooplankton (E. Jermolajew, unpublished) has shown that the amount of microcrustacea available to fish for food increases from Minas Channel to the Shubenacadie estuary and it consists of estuarial species such as *Acartia tonsa* and *Pseudodiaptomus coronatus* that cannot have come from outer waters. Tows made in 1951, showed the following numbers of zooplanktonts: Minas Channel with water as deep as 57 fathoms, 4,700 and 5,700; Minas Basin (southern bight) with water as deep as 13 fathoms, 14,800 and 25,400; Cobequid Bay with water as deep as 10 fathoms, 50,200 and 89,900; Shubenacadie estuary with water as deep as 3 fathoms, 253,800.

However, a very shallow estuary with heavy tides and turbulent water is not necessarily more productive than a deep one. The inner, shallow part of the Petitcodiac estuary of the northern branch of the

Bay of Fundy yields practically no fish. The deeper outer part has a fishery for salmon and shad, which are concentrated toward its head where the salter water flowing in below begins to be thoroughly mixed with the outflow of less salt water. The shallow inner part, which is twenty miles long, is traversed by a tidal bore. The breaking tidal wave churns up the estuarial water with the silt of the bottom to make a heavy suspension of clay, which buoys the animals up to the surface where they become prey for birds. What plants are produced in it has not been investigated, but it presents a distinct hazard for animals, and those that enter it, such as salmon, pass through it very slowly owing to the great turbulence.

SUMMARY

Striking differences in the amounts of fish taken in different parts of the Bay of Fundy reflect differences in local abundance of fish. These differences receive explanation from knowledge of where the plants and animals which make up the food chain are grown and of how they are transported by the circulation of the water.

Great rise and fall of the tide in the bay makes the water turbulent and hence, as a whole, both turbid and rich in nutrient salts for plant growth from updraft of bottom water and sediment. The turbidity and the turbulence largely prevent growth of floating plants, which fail to remain long in the thin surface layer which alone has sufficient light. The turbulence mixes river water, which comes in largest amount from the St. John River through Saint John harbour, with many times its volume of sea water, and the relatively light mixture, which is rich in nutrient salts, flows out into the Gulf of Maine. Where it spreads near the surface it yields a heavy and continuous growth of phytoplankton in accordance with temperature and light. The animals, mainly microcrustacea, which feed upon the phytoplankton are carried into the bay in the deep return current and in the general counter-clockwise circulation of gulf and bay, in accordance with the depths at which they live.

The heavy discharge of the St. John River and of the rivers tributary to Passamaquoddy Bay, when mixed with much sea water, ensures, through return currents, more or less steady and large supplies of the microcrustacea for the food of fishes in the outer part of the Bay of Fundy. The Passamaquoddy mechanism, a complex circulation of the water, concentrates the herring from the late fry stage onward, and the microcrustacea for their food, in that region. It also segregates the sizes of herring, makes the microcrustacea available near the surface where the herring live, and largely concentrates the small herring and the zooplankton suitable for their food far inland, where they are relatively free from enemies and from competition for food.

The zooplankton from the Gulf of Maine is carried into the inner part of the Bay of Fundy but slowly, owing to the very great turbulence, and enters mainly along the Nova Scotian shore. Such food for fish decreases in amount going inward and becomes poor through lack of phytoplankton for food. It fails to enter Minas Channel, which is most barren of plankton.

With decreasing depth from Minas Channel to the Shubenacadie estuary, conditions for growth of phytoplankton improve, since turbulence cannot take these organisms far from the light near the surface. In accordance with this, the planktonic microcrustacea, consisting of locally produced estuarial forms, increase in amount and so do the fish. Although very shallow, the inner part of the Petitcodiac estuary is very barren because it is traversed by a tidal bore, which through suspended silt buoys animals to the surface to become prey for birds.

The local abundance of fish which determines large catches is to a considerable extent attributable merely to the transport and concentration of the fish by the circulation of water.

REFERENCES

CLARKE, G. L. (1939). The utilization of solar energy by aquatic organisms. Publ. Amer. Ass. Adv. Sc., **10**: 27.

FISH, C. J. (1936a). The biology of *Calanus finmarchicus* in the Gulf of Maine and Bay of Fundy Biol. Bull., **70**: 118.

——— (1936b). The biology of *Pseudocalanus minutus* in the Gulf of Maine and Bay of Fundy. Biol Bull, **70**: 193.

——— (1936c). The biology of *Oithona similis* in the Gulf of Maine and Bay of Fundy. Biol. Bull., **71**: 168.

FISH, C. J. and JOHNSON, M. W. (1937). The biology of the zooplankton population in the Bay of Fundy and Gulf of Maine with special reference to production and distribution. J. Biol. Bd. Can., **3**: 189.

GRAHAM, M. (1936). Investigations of the herring of Passamaquoddy and adjacent regions. J. Biol Bd. Can., **2**: 95.

GRAN, H. H and BRAARUD, T. (1935). A quantitative study of the phytoplankton in the Bay of Fundy and the Gulf of Maine including observations on hydrography, chemistry and turbidity. J. Biol. Bd. Can., **1**: 279.

HACHEY, H. B. (1935). Tidal mixing in an estuary. J. Biol. Bd. Can., **1**: 171.

HUNTSMAN, A. G. (1918). The effect of the tide on the distribution of the fishes of the Canadian Atlantic coast. Trans. Roy. Soc. Can., 3rd ser., **12**(sec. IV): 61-7.

——— (1923). Natural lobster breeding. Bull. Biol. Bd. Can., **5**: 1.

——— (1928). The Passamaquoddy bay power project and its effect on the fisheries. Saint John, N.B. Pp. 1-45.

——— (1934). Herring and water movements. In James Johnstone Memorial Volume, pp. 81-96. Liverpool.

——— (1938). International Passamaquoddy fishery investigations. J. Cons. Inter. Explor. Mer., **13**: 357.

HUNTSMAN, A. G. and REID, M. E (1921). The success of reproduction in *Sagitta elegans* in the Bay of Fundy and the Gulf of St. Lawrence. Trans. Roy. Can. Inst., **13**(2): 99.

JOHNSTONE, J. (1908). Conditions of life in the sea Cambridge: Cambridge University Press. Pp. 1-332.

UNITED NATIONS, FOOD AND AGRICULTURAL ORGANIZATION (1950). Yearbook of fisheries statistics, 1948-49. Pp. 1-312.

WATSON, E. E. (1936). Mixing and residual currents in tidal waters as illustrated in the Bay of Fundy. J. Biol. Bd Can., **2**: 141.

WRIGHT, N. (1929). In J. J Cowie: Report on the work of the Biological Board for 1928-29 Ann Rep. Fisher. Br, Dep Mar. Fisher. Can, **62**: 123.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVI : SERIES III : JUNE, 1952
SECTION FIVE

Thyroid Function in Some Anadromous and Landlocked Teleosts

WILLIAM S HOAR
Presented by W. A CLEMENS, F R S C.

INTRODUCTION

ANADROMOUS teleost fishes of many species, although usually migrating to sea as juveniles, may complete their entire life cycle in fresh water. This frequently occurs in lake regions, remote from the sea, where the fish are referred to as "landlocked." The members of some anadromous species migrate soon after hatching; others have a prolonged juvenile, stenohaline freshwater phase. The smelt (*Osmerus mordax*), alewife (*Pomolobus pseudoharengus*), chum (*Oncorhynchus keta*), and pink salmon (*O. gorbuscha*) are representatives of the first group, while the sockeye (*O. nerka*), coho (*O. kisutch*), and Atlantic salmon (*Salmo salar*) belong to the second. This latter group shows a distinct physiological transformation prior to seaward migration (Hoar, 1951).

The success of the different species when landlocked is evidently quite variable (Table I). At one extreme is the smelt which grows as

TABLE I

LENGTHS OF ANADROMOUS FISH MATURING IN MARINE OR FRESHWATER HABITATS

(Measurements given are for the older age groups but are not necessarily the maximum lengths)

Species	*Length* (mm) Marine	Freshwater	*Authority*
Salmo salar	480*	350	Wilder (1947)
Oncorhynchus nerka	820	200–400	Carl and Clemens (1948)
Pomolobus pseudoharengus	258	145	Pritchard (1929)
Osmerus mordax	150–250		McKenzie (1946); Vladykov and McKenzie (1935)
		150–250	Dymond (1944); Van Oosten(1944)

*Grilse or salmon maturing after 1 year in the sea. Older fish may reach 1200 mm. (Carl and Clemens, 1948).

well in lakes as in the ocean. At the other extreme, the alewife matures at a much smaller size in lakes and shows a spectacular annual mortality (Pritchard, 1929). In general, landlocked teleosts seem to grow more slowly than the marine forms but are not subject to the annual mortality shown by the alewife.

Several writers have emphasized the importance of thyroid hormone in growth regulation of fishes. In addition, there is an evident relationship between osmotic regulation and thyroid activity. The literature has recently been reviewed (Hoar, 1951) and need not be discussed in detail here. It is sufficient to note that marine fish placed in dilute sea water show increased thyroid activity (Leloup, 1948; Olivereau, 1948) and that some anadromous fish retained in fresh water beyond the natural time of seaward migration develop hyperplastic thyroids (Hoar and Bell, 1950). The latter observation is not unexpected if the first is true, since the seaward migrating fish must have many of the physiological characteristics of a marine fish. The biochemical changes which occur in Atlantic salmon prior to seaward migration have now been studied in some detail. The elevation in blood iodine (Fontaine and Leloup, 1950a), in blood copper (Fontaine, 1948), and in degree of unsaturation of body fats (Lovern, 1934) all combine to change the tissues of the freshwater salmon parr toward the marine salmon at the time of smolt transformation. These investigations suggest, then, that thyroid hormone enters into the metabolic chains involved in the elimination of water and that the anadromous teleost making a prolonged stay in fresh water is under added osmotic stress which demands additional thyroid hormone. Such a suggestion agrees with current views on the metabolic role of thyroid hormone in other vertebrates since the elimination of water against an osmotic gradient requires a considerable expenditure of energy.

Changes in thyroid activity of alewives and smelt from coastal and landlocked environments are described in this paper as further evidence of the functions of the thyroid hormone in anadromous teleosts. Conclusions are based on histological evidence. It is recognized that the variations in thyroid function are reflected in well-marked changes in the microscopical picture of the gland (Ham, 1950). The follicles of a quiescent thyroid contain an abundance of stored colloid which is homogeneous and brightly acidophilic. The follicular colloid in the stimlulated gland becomes progressively more granular and basophilic owing to enzymatic hydrolysis (de Robertis, 1949). It may eventually disappear entirely. The vacuoles frequently observed at the periphery of the follicles, although artefacts in themselves (de Robertis, 1949), are further evidence of stimulation. The epithelial cells of the stimulated thyroid increase in number and height. Increased vascularity is also characteristic and colloid material is often observed in the vascular channels.

MATERIALS AND METHODS

The fish whose thyroids are described in this paper came from a number of different places (Table II). A. G. Huntsman collected juvenile alewives (*Pomolobus pseudoharengus*) from the Grand Lake region of Nova Scotia. Adult alewives were obtained from the Kennebecasis estuary, New Brunswick, through the co-operation of L. R. Day and J. C. Medcof. R. A. McKenzie provided juvenile and mature smelt (*Osmerus mordax*) from the Miramichi River and estuary in New Brunswick. The landlocked alewives and smelt from the Great Lakes were contributed by F. E. J. Fry and J. J. Graham. The co-operation of all those who have made the study possible is gratefully acknowledged.

Bouin's picric-acid-formol-acetic-acid mixture was the fixative of choice. However, some of the fish used were taken from routine formalin collections made for other purposes. This is particularly true of the fish from Ontario. Thyroids were embedded in paraffin, sectioned serially at 7μ or 10μ and stained with Harris' hematoxylin and eosin. Merva K. Cottle made most of the preparations but Dorothy Hamilton also spent many hours on the project. It is a pleasure to thank them for the care and skill with which they put so many thyroids on glass. Financial assistance for this part of the project was obtained from a University Research Grant.

RESULTS

Alewives

Juvenile seaward migrants from the Atlantic coast. Thyroids of migrants (25 to 65 mm. total length), collected during the latter part of July, are uniformly quiescent. In the smaller fish the gland is incompletely developed. Small, spherical, differentiating follicles are scattered widely. The epithelium is squamous or low cuboidal; the colloid brightly eosinophilic, highly refractile and abundant (Fig. 1). Fish 50 to 60 mm. in length have well-differentiated thyroids with the glandular tissue characteristically distributed about the ventral aorta. The follicles are larger, made up of cuboidal cells, and contain abundant, eosinophilic colloid (Fig. 2). The texture varies from highly refractile to slightly granular. Colloid is sometimes conspicuous in the lymphatics.

Specimens collected from the Lower Rawdon River a month later

TABLE II
THYROIDS EXAMINED HISTOLOGICALLY

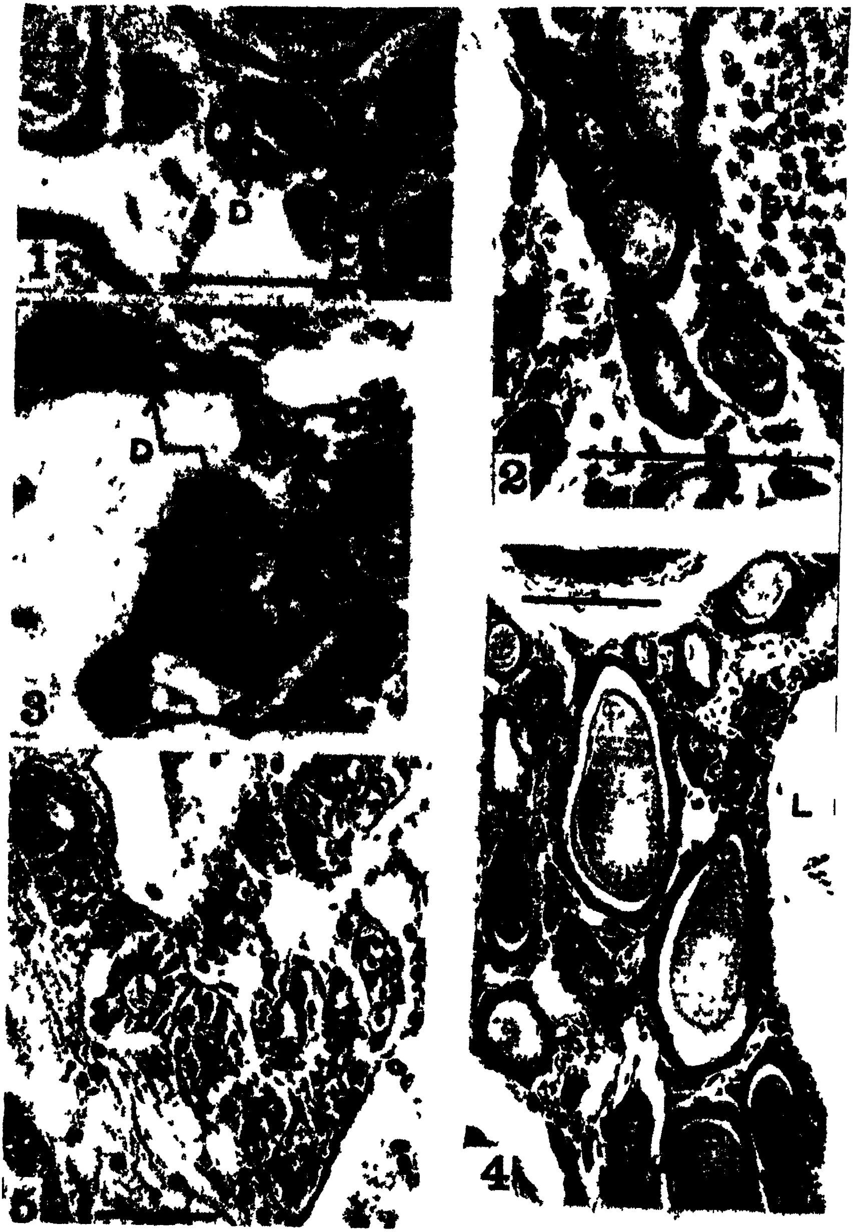
Place of capture	*Date*	*Maturity*	*Length of fish* (mm.)	*Number*
ALEWIVES				
Fletcher Dam, N.S.	July 24–6, 1949	seaward migrants	25–65	15
Lower Rawdon River, N.S.	Aug. 22, 1949	" "	95–100	4
Milledgeville, N.B.	Feb. 1951	maturing adults, estuary	300–350	4
Hamilton Beach, Ont.	May 1950	adults, prespawning	135–190	8
Bay of Quinte, Ont.	Aug. 1950	post-spawning adult	150	1
" " " "	May 1950	mortality victim	172	1
Gananoque River, Ont.	June 1950	adult	155–165	2
SMELT				
Miramichi, N.B.	July–Sept. 1942	seaward migrants	30–50	10
South Bay, Ont.	July 1951	juveniles of the year	25–30	3
Miramichi estuary	May 7, 1951	immature yearlings	100–105	3
South Bay, Ont.	April 1950	spawning run	140–165	7
" " "	April 28, 1951	" "	140–165	5
Miramichi River	May 9, 1949	spawning and spent	140–198	7
Miramichi estuary	Sept. 8, 1949	adults	170–185	2

are about twice as large (95 to 100 mm.). The thyroids are more active than those of the younger fish but do not show the marked activity characteristic of seaward migrating salmon smolt of comparable size (Hoar, 1939). Proliferation of new thyroid follicles, however, is apparent (Fig. 3) and the thyroid mass is evidently extending. The epithelium is cuboidal, at times approaching a columnar type. In places the colloid is almost completely withdrawn but more often it is abundant, homogeneous, and eosinophilic (Fig. 4). The follicles are larger at this stage (25 to 50μ in contrast to 15 to 30μ in the 50 mm. fish) but this increase is not out of proportion to the increased size of the fish. The thyroids are similar to those of migrating Pacific salmon found in coastal areas (Hoar and Bell, 1950). There is no marked increase in activity associated with the seaward migration.

Sexually mature adults from the Atlantic coast. Thyroids from fish in this group are characterized by the mild state of activity which is apparently normal for sexually maturing teleosts (Fontaine *et al.*, 1948; Fontaine et Leloup, 1950b). The epithelium varies from cuboidal (Fig. 6) to low columnar (Fig. 5). Many follicles contain refractile, eosinophilic colloid riddled with peripheral vacuoles. These vacuoles are not always present, however, and the colloid may be more basophilic. In many areas the colloid is absent, and the smaller follicles of columnar epithelium contain only a fine reticulum (Fig. 5). The two types of follicles are present in different parts of the same gland and frequently occur in the same histological section. The gland is active but in no case hyperplastic or exhausted.

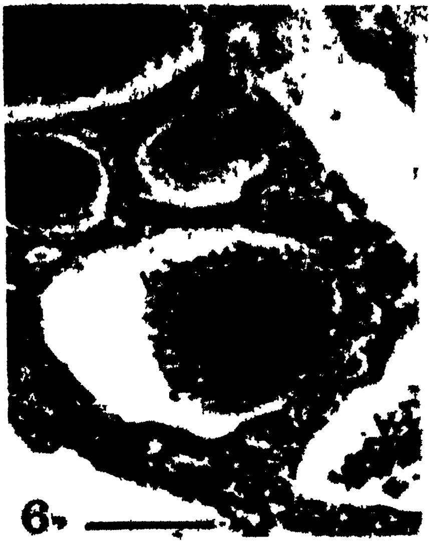
Mature alewives from Lake Ontario. These thyroids differ markedly from those of the Atlantic coast fish just described. The glands are extremely hyperplastic and in many cases appear to be exhausted. Empty follicles are packed in masses about the blood vessels and lymphatics. They extend into the connective tissue spaces around the muscle masses and into the cavities of the basibranchial bones (Fig. 7). Colloid-containing follicles were located in only five of twelve thyroids examined in serial sections. One thyroid was pathological. In this gland organized follicles were absent and the thyroid area was infiltrated with masses of lymphocytes (Fig. 9). No particular significance can be attached to this since a pathological gland was also found in one of the larger juveniles from the Lower Rawdon River (Fig. 10). Similar pathologies have been previously described in thyroids from fish (Gaylord and Marsh, 1912).

Large masses of empty follicles are the most characteristic feature of the thyroids from the landlocked alewives. The epithelium is uniformly cuboidal or low columnar and never assumes the high

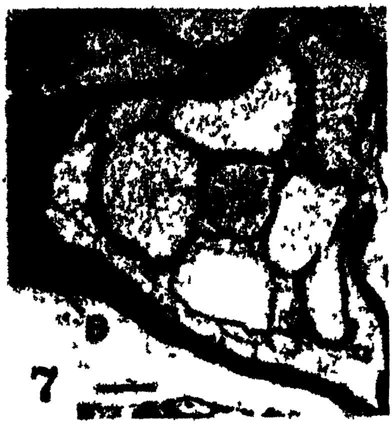


Legend for all plates *B*, bone, *BV*, blood vessel, *C* colloid, *CT*, cartilage, *D*, differentiating follicles, *L*, lymphatic, *V*, peripheral vacuoles

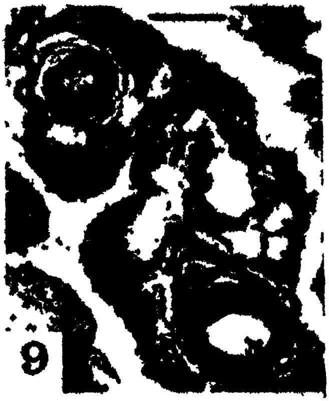
PLATE I —Photomicrographs of sections of thyroid glands of alewives from the Atlantic coast, locations given in Table II Horizontal line on each figure is 50μ FIGURE 1 —25 mm fry Small differentiating follicles near well-formed colloid-containing follicles FIGURE 2 —55 mm fry FIGURE 3 — 100 mm juvenile. FIGURE 4 —98 mm juvenile FIGURE 5 —Maturing adult from estuary Follicles contain only fine basophilic reticulum



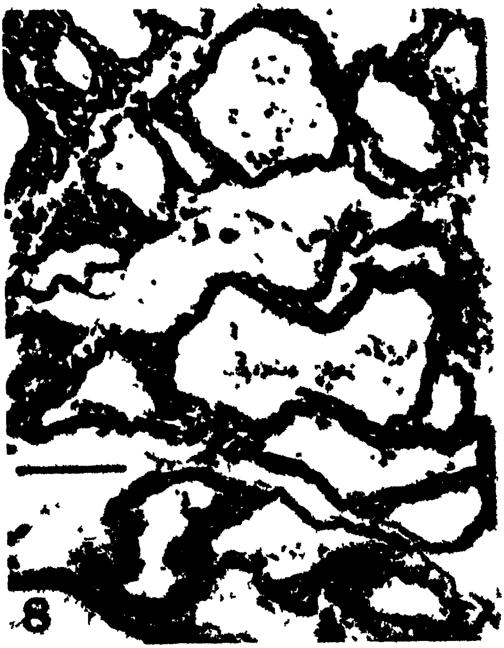
6



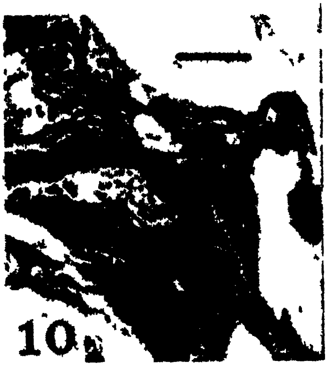
7



9



8



10

PLATE II.—Photomicrographs of sections of thyroid glands from alewives. Horizontal line on each figure is 50μ. FIGURE 6.—Maturing adult from Kennebecasis estuary. Follicular epithelium is low and colloid shows peripheral vacuoles. FIGURE 7.—Mature adult, Hamilton Beach, Ont. Seven empty follicles almost surrounded by basibranchial bone. FIGURE 8.—Same as Fig. 7 showing numerous empty follicles. FIGURE 9.—Mature fish, Bay of Quinte, Ont. Mass of tissue from pathological thyroid runs diagonally across field. FIGURE 10.—95 mm. juvenile from Lower Rawdon River, N.S. Masses of unorganized cells in pathological gland.

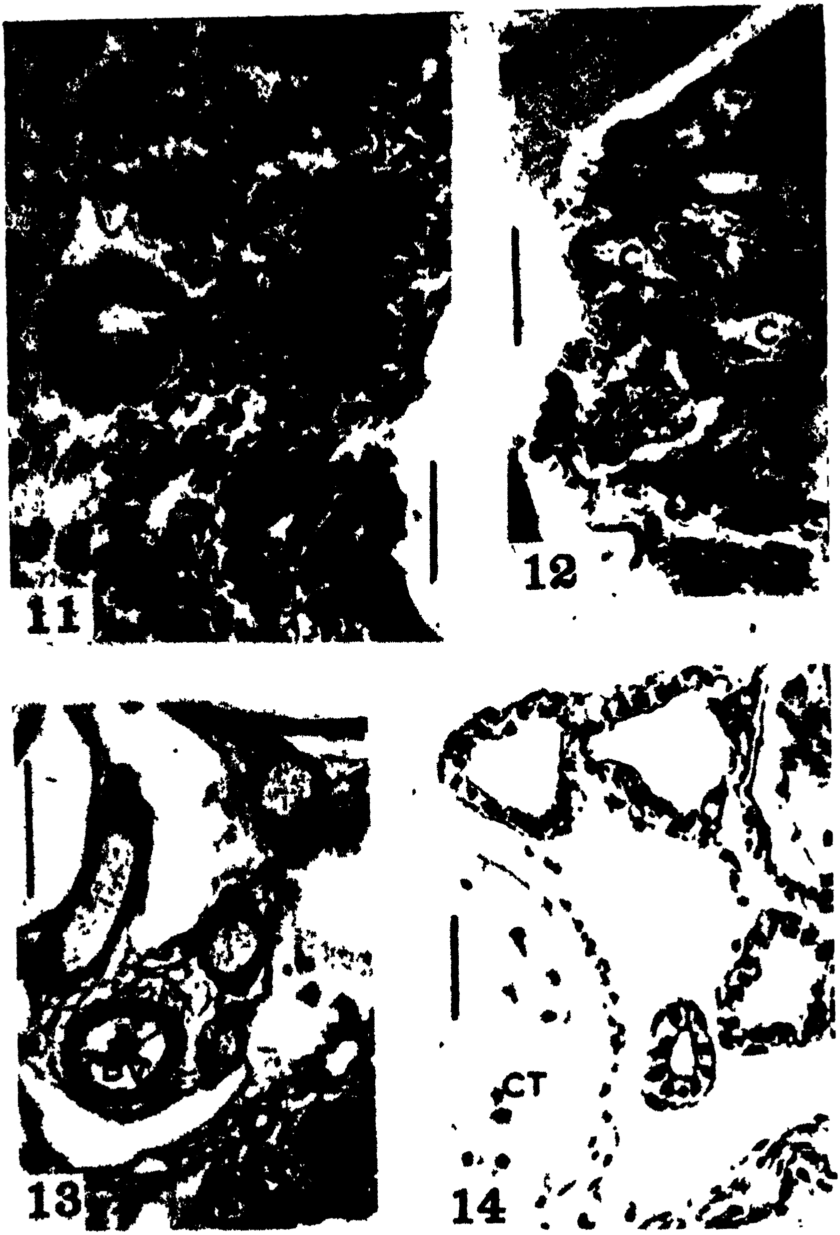


Plate III.—Photomicrographs of sections of thyroid glands Vertical line on each figure is 25μ Figure 11 — Mature alewife from Gananoque River, June 1950 Follicles with high cuboidal or columnar epithelium sometimes contain eosinophilic colloid. Figure 12.—Same as Fig. 11. Colloid in follicles and lymphatics is eosinophilic. Figure 13 —30 mm. smelt from Miramichi River. Granular basophilic colloid in follicles. Figure 14.—30 mm. smelt from South Bay, Ont. Four empty follicles.

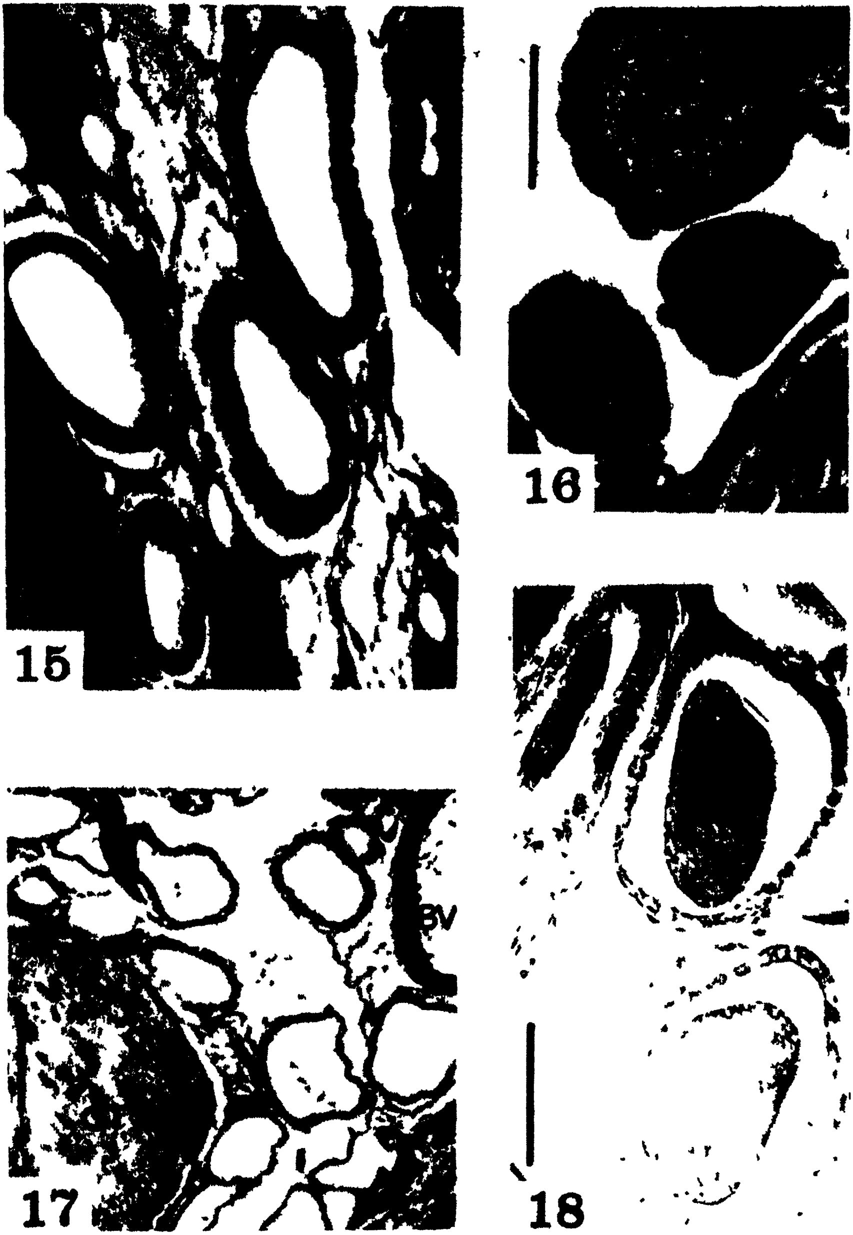


PLATE IV.—Photomicrographs of sections of smelt thyroid glands from Miramichi River and estuary. FIGURE 15.—50 mm fry. Empty follicles, colloid in lymphatics. Vertical indicator line is 25μ. FIGURE 16.—50 mm fry Basophilic granular colloid. Vertical indicator line is 25μ FIGURE 17.—Immature yearling smelt (105 mm.) from tide water. Vertical indicator line is 100μ. FIGURE 18 — 170 mm adult male from estuary in September. Cuboidal epithelium, acidophilic colloid with some peripheral vacuoles. Vertical indicator line is 50μ.

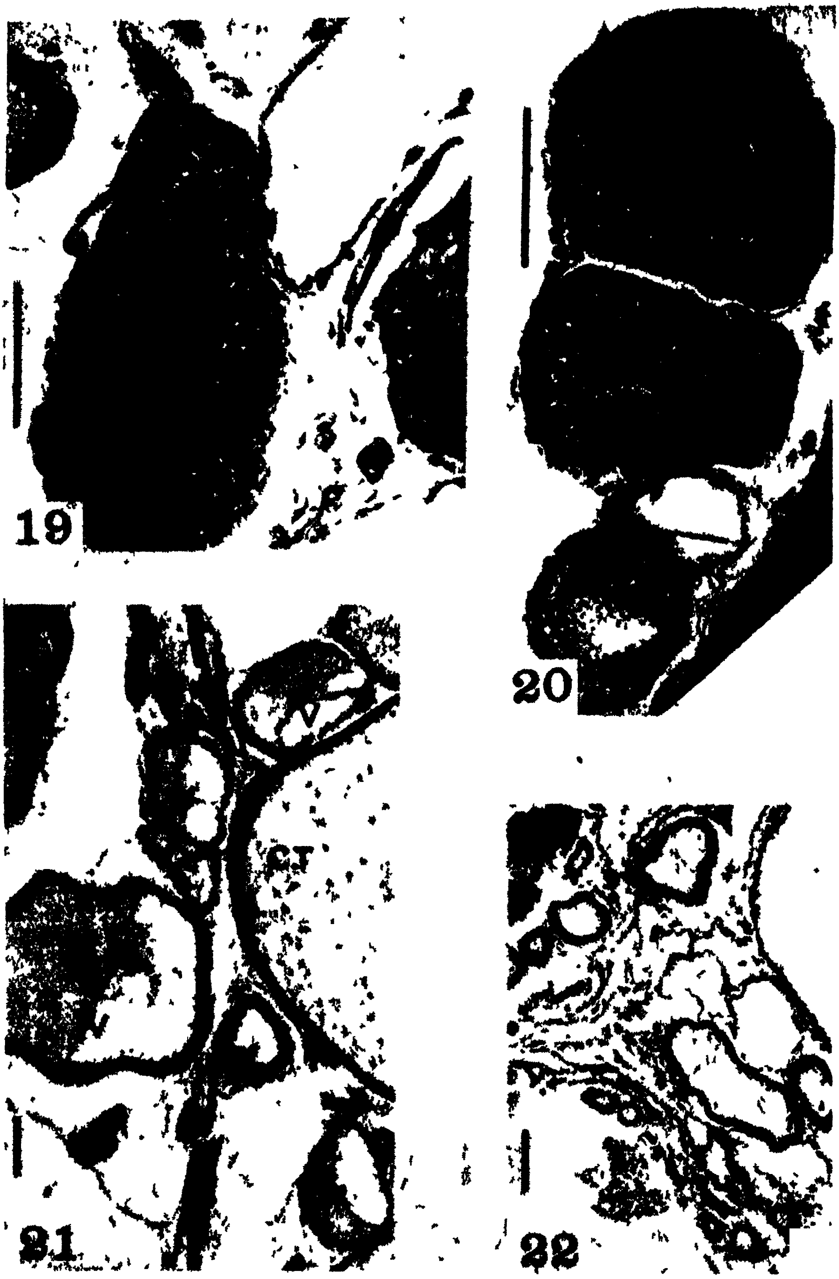


PLATE V.—Photomicrographs of sections of thyroid glands from smelt. Vertical line on each figure is 50μ. FIGURE 19.—175 mm. female, spent and leaving spawning grounds May 1949, Miramichi River, N B FIGURE 20.—165 mm male about spent and leaving spawning grounds May 1949, Miramichi River, N B. FIGURES 21 and 22.—Mature smelt from spawning run, South Bay, Ont , April 28, 1951.

columnar form often characteristic of very active thyroids. Follicles are large and irregular in shape indicating an overgrowth of the epithelial cells. The absence of stainable colloid is a striking feature. These characteristics are illustrated in Fig. 8.

As previously mentioned, colloid-containing follicles were present in about 50 per cent of the glands studied. One thyroid examined, from an adult in the post-spawning condition, contained many such follicles but the other four showed only a relatively insignificant number. These colloid-containing follicles are usually small (25 to 35μ in diameter), lined with high cuboidal or columnar epithelium, and contain brightly acidophilic colloid (Fig. 11). They are sometimes isolated but more often in small groups. The presence of colloid in the lymphatics is a characteristic feature (Fig. 12).

Smelt

Juvenile underyearling smelt. Smelt fry of 25 to 30 mm. total length were available from coastal areas and from the Great Lakes. In both cases the thyroid gland is differentiating and has not yet attained its final distribution. These developing thyroids all show evidence of activity. This activity, however, is more marked in the landlocked fish. Thyroids from the coastal smelt fry are characterized by cuboidal epithelium, some basophilic follicular colloid, and an abundance of colloid in the lymphatics; thyroids from the Great Lakes smelt fry show high cuboidal epithelium and an absence of follicular colloid. Figs. 13 and 14 show the contrast.

Larger juveniles (40–50 mm.) are available only from the Miramichi. The thyroid glands are larger. Although stored colloid is usually more abundant it may be entirely absent (Fig. 15). When present, it stains slightly basophilic (Fig. 16). Colloid is conspicuous in the lymphatics and blood vessels (Fig. 15). These mildly active thyroids do not differ greatly from those of the smaller fry.

Immature yearling smelt from the estuary. Thyroids of these fish are similar to those of the seaward migrating fry. The relatively large follicles are made up of a cuboidal epithelium and are either empty or contain a somewhat basophilic colloid with prominent peripheral vacuoles (Fig. 17). The epithelium is often folded. Colloid is conspicuous in the blood vessels and lymphatics.

Spawning smelt from the Miramichi River. The thyroid follicles of the spawning smelt are bounded by columnar or high cuboidal epithelium and filled with brightly acidophilic, highly refractile colloid (Figs. 19 and 20). Peripheral vacuoles are rarely seen and colloid is not evident in the vascular channels. Colloid storage, therefore, seems characteristic of spawning Atlantic smelt as well as salmon (Hoar, 1939; Fontaine *et al.*, 1948).

It is interesting that the follicular epithelium of male fish at spawning time is high columnar (Fig. 20), that of the females low columnar or high cuboidal (Fig. 19). This contrast between the thyroids of the two sexes was consistent in the three males and four females examined. Empty follicles were occasionally found in the thyroids of female smelt and the lower epithelium, contrary to the usual situation (Ham, 1950), seems to indicate a more active gland in these fish.

Post-spawning smelt from the Miramichi estuary. These fish were collected in September and had probably spawned during the previous spring. The conspicuous vacuoles and lower epithelium may indicate a slightly more active condition in the male gland illustrated in Fig. 18. However, the general picture is one of colloid storage and quiescence.

Spawning smelt from South Bay, Ontario. In contrast to the colloid storage described in the spawning Miramichi smelt, the fish from South Bay have active glands. The follicular epithelium is cuboidal or high cuboidal. Some follicles are empty, many contain granular basophilic material (Fig. 22). Acidophilic refractile colloid, when present, is conspicuously eroded by peripheral vacuoles (Fig. 21).

DISCUSSION

Thyroids of juvenile alewives and smelt from the Atlantic coast are quiescent or mildly active at the time of seaward migration. They are similar to the glands of chum and pink salmon migrating to sea during their first spring and summer (Hoar and Bell, 1950). Although alewives and smelt do not have a prolonged freshwater feeding period and show a distinct smolt transformation, their seaward migration may extend over a period of several months. Thyroids are slightly more active in the later migrants. However, even the larger alewives, which are comparable in size to salmon smolt, do not have markedly active thyroids.

Thyroid stimulation seen in the sexually maturing alewives from the estuary and the colloid storage evident in thyroids of spawning and spent smelt from the Miramichi River correspond to conditions which have been previously described in Atlantic salmon at comparable times in their life cycle (Fontaine *et al.*, 1948). However, it should be noted that the detailed histological picture of the resting gland in spawning smelt differs from that of salmon (Hoar, 1939) and from the usual picture of thyroid involution (Ham, 1950). The high cuboidal or columnar epithelium, in contrast to the usual low cuboidal or squamous variety, is surprising. In spite of this irregularity, however, the glands are quiescent as evidenced by: (*a*) follicles packed with intensely eosinophilic homogeneous colloid, (*b*) absence of peripheral follicular vacuoles, and (*c*) lack of colloid in the lymphatics.

The most interesting findings in the present study arise from comparison of the coastal and landlocked fish. The thyroids of the landlocked fish are always more active than those of coastal fish in a comparable stage of development. Both juvenile and adult smelt were compared. Figs. 13 and 14 for juveniles and Figs. 19, 20, 21, and 22 for adults show the contrast. Only mature alewives were obtained from both environments. The marked difference is evident in Figs. 6 and 8. Further, this hypertrophy is so great in the case of the landlocked alewife that the gland has every appearance of exhaustion.

Heightened thyroid activity in anadromous fish from landlocked environments is not unexpected. As previously discussed, the elimination of water seems to require thyroid hormone and added osmotic stress may be expected when the anadromous fish is retained beyond the time of natural migration. The contrast between landlocked alewives and smelt is, however, particularly interesting. The alewife thyroid is enlarged, hyperplastic, and shows an exhaustion of colloid; the smelt thyroid is of normal size and, although active, contains an abundance of colloid. This contrast may depend upon different abilities in the two species to limit the amount of water in the tissues or to excrete water. At any rate, it is suggested that the alewife, owing to some difference in the biochemical constitution of its tissues, is under greater osmotic stress than the smelt. Another possibility, which should not be ignored, is a difference in the ability ot the two species to utilize the relatively small amounts of iodine available in the Great Lakes region. Many years ago, Marine and Lenhart (1910) examined thyroids from a variety of Great Lakes fish (not including smelt or alewife) and found mildly active glands in several species. They concluded that fish, as well as man, may be affected by the supply ot iodine in regions of endemic goiter. However, the mild activity described by Marine and Lenhart (1910) is no greater than the normal seasonal change shown by some fish (Hoar, 1939) or than that which precedes sexual maturation in several species (Fig. 5 and Fontaine *et al.*, 1948).

The relatively slow rate of growth in landlocked alewives may be related to the thyroid mechanism. Smelt reach essentially the same size in coastal and landlocked environments but alewives are much smaller when landlocked (Table I). The growth regulating function of the thyroid hormone is recognized. If the supply of hormone is inadequate for both osmotic regulation and growth the latter will probably be reduced. In man, inadequate supplies of hormone are known to produce dwarfing (cretinism) and experimental work suggests that a similar situation will occur in fish (Hoar, 1951).

Finally, it is suggested that the annual mortality of the Great Lakes alewives may be related to an exhausted thyroid. If, as has been argued, thyroid hormone is involved in the elimination of water, an exhaustion of the thyroid mechanism may lead to death from water intoxication. At least three factors may operate to increase the demand for thyroid hormone at the time of the annual mortality. Several species of fish normally show in the spring thyroid stimulation which is unrelated to sexual maturation (Hoar, 1939). Changes in illumination, acting by way of the pituitary, or temperature may be responsible but the exact mechanism has not been established. In addition, thyroid activity seems always to be associated with the early stages of sexual maturation and, finally, the seasonal elevation in water temperature may increase the osmotic load through a direct effect of temperature on permeability. This extra load of water will create additional demands for hormone. Any one of these factors may be sufficient to overload an already severely taxed thyroid mechanism.

SUMMARY

1. The cycle of thyroid activity found in Atlantic alewives and smelt is characteristic of anadromous fish which do not have a prolonged freshwater phase. Thyroids of seaward migrants are quiescent or mildly active. Maturing adults have active glands. Spawning adults show storage of colloid.

2. Thyroids of landlocked alewives and smelt are always more active than those of the same fish taken in coastal areas. In the smelt the gland is active but not hyperplastic; in the alewife it is extremely large, hyperplastic, and exhausted.

The relatively slow growth rate of the alewife and the periodic mortalities may be related to this condition of thyroid exhaustion.

REFERENCES

Carl, G. C. and Clemens, W. A (1948). The fresh-water fishes of Biritish Columbia. Provincial Museum, Handbook no 5. Victoria.

De Robertis, E (1949). Cytological and cytochemical bases of thyroid function. Ann. N.Y. Acad. Sci. **50**: 317–35

Dymond, J. R. (1944). Spread of the smelt (*Osmerus mordax*) in the Canadian waters of the Great Lakes. Can. Field-Nat. **58**: 12–14.

Fontaine, M. (1948). Du rôle joué par les facteurs internes dans certaines migrations de poissons. J. Conseil **15**: 284–94.

Fontaine, M., Lachiver, F., Leloup, J. et Olivereau, M. (1948). La fonction thyroïdienne du Saumon (*Salmo salar* L.) au cours de sa migration reproductrice. J Physiol. (Paris). **40**: 182–4.

FONTAINE, M. et LELOUP, J. (1950a). L'iodémie du jeune Saumon (*Salmo salar* L.) en eau douce. C.R. Acad. Sci. **231**: 169–71.

——— (1950b). Sur l'iodémie de deux Téléostéens migrateurs Potamotoques *Salmo salar* L. et *Alosa alosa* L. au début de leur montée reproductrice. C.R. Acad. Sci. **230**: 775–7.

GAYLORD, H. R. and MARSH, M. C. (1912). Carcinoma of the thyroid in the salmonoid fishes. Bull. U.S. Bur. Fish. **32**: 367–524.

HAM, A. H. (1950). Histology. Philadelphia: Lippincott.

HOAR, W. S. (1939). The thyroid gland of the Atlantic salmon. J. Morph. **65**: 257–95.

——— (1951). Hormones in fish. Univ. Toronto Stud. Biol. 59, Pub. Ont. Fish Res. Lab. **71**: 1–51.

HOAR, W. S. and BELL, G. M. (1950). The thyroid gland in relation to the seaward migration of Pacific salmon. Can. J. Res. D**28**: 126–36.

LELOUP, J. (1948). Influence d'un abaissement de salinité sur la cuprémie de deux Téléostéens marins: *Muraena helena* L., *Labrus bergylta* Asc. C.R. Soc. Biol. (Paris) **142**: 178–9.

LOVERN, J. A. (1934). Fat metabolism in fishes. V. The fat of the salmon in its young freshwater stages. Biochem. J. **28**: 1961–3.

MARINE, D. and LENHART, C. H. (1910). On the occurrence of goitre (active thyroid hyperplasia) in fish Johns Hopkins Hosp. Bull. **21**: 95–8.

MCKENZIE, R. A. (1946). The smelt fishery of northeastern New Brunswick. Bull. Fish. Res. Bd. Can. **70**: 1–20.

OLIVEREAU, M. (1948). Influence d'une diminution de salinité sur l'activité de la glande thyroide de deux Téléostéens marins: *Muraena helena* L., *Labrus bergylta* Asc. C.R Soc. Biol. (Paris) **142**: 176–7.

PRITCHARD, A. L. (1929). The alewife (*Pomolobus pseudoharengus*) in Lake Ontario. Univ. Toronto Stud. Biol. 33, Pub. Ont Fish. Res. Lab. **38**: 39–54.

VAN OOSTEN, J. (1947). Mortality of smelt, *Osmerus mordax* (Mitchell), in Lakes Huron and Michigan during the fall and winter of 1942–1943. Trans. Amer. Fish. Soc. **74** (1944): 310–37.

VLADYKOV, V. D. and MCKENZIE, R A. (1935). The marine fishes of Nova Scotia. Proc. N.S. Inst. Sci. **19**, part 1: 17–113.

WILDER, D. G. (1947). A comparative study of the Atlantic salmon, *Salmo salar* Linneaus, and the lake salmon, *Salmo salar sebago* (Girard). Can. J. Res. D**25**: 175–89.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVI : SERIES III : JUNE 1952
SECTION FIVE

"Even-year" and "Odd-year" Pink Salmon Populations

FERRIS NEAVE
Presented by J. L. HART, F.R.S.C.

SINCE pink salmon (*Oncorhynchus gorbuscha*), to the best of our current knowledge, invariably mature and die at the end of the second year of life, it has long been recognized that the adult runs appearing in any two consecutive years represent entirely separate populations of the species. Since these stocks for the most part occupy the same regions and spawn in the same watercourses, special opportunities are provided for comparing the status and fortunes of two populations possessing similar constitutional and geographic opportunities but entirely prevented from interbreeding

Particular interest attaches to well-known cases in which the disparity in abundance between the stocks is so great that years of immensely productive fishing alternate regularly with years which yield insignificant returns from the same waters.

This situation has sometimes led to the belief that the existence of a very large stock in one series tends to suppress the population of the other series—a view which has been more fully developed in relation to a somewhat similar phenomenon of "dominance" exhibited by the sockeye (*O. nerka*) populations of the Fraser River watershed (Ricker, 1950).

Whether or not the causes of population disparity in pink salmon can be immediately elucidated, a decision about the independence or otherwise of each population with respect to the other would in itself be helpful to the conservationist. If it were shown that the existence of two large populations in the same waters is biologically incongruous, fisheries management would have to work within the limitations of this framework. If on the other hand there is no fundamental incompatibility, efforts can be directed towards discovering and removing the causes of weak stocks.

In evaluating the evidence bearing on this problem it is pertinent to survey the available information concerning the geographic status of pink salmon belonging to the two great branches of the species, that is, fish maturing in "even" years and fish maturing in "odd" years. For convenience, these two kinds of pink salmon are designated herein as "E fish" and "O fish".

RELATIVE ABUNDANCE OF E- AND O- PINK SALMON

1. *In British Columbia*

Past records of the abundance of adult pink salmon (that is, "catches" and "escapements") for the numerous administrative areas of the British Columbia coast cannot be regarded as accurate in an absolute sense. They are undoubtedly adequate, however, to show the direction of major changes in abundance from year to year or to reveal marked discrepancies between a series of even years and a series of odd years.

Table I presents certain such estimates of catches for areas of the northern and central coastal regions of British Columbia. These areas are defined in Fig. 1. For the southern portions of the coastline, catch figures cannot readily be used as an indication of the condition of the stocks "belonging" to individual areas, since many of the fish caught

TABLE I

RELATIVE ABUNDANCE OF E AND O PINK SALMON, IN NORTHERN B.C., AS INDICATED BY REPORTED ANNUAL CATCHES

Area	Period	*Average catch (wt)* E	 O	*Ratio* E:O
North Queen Charlotte Is.	1930-1949	45,994	224	205:1
South Queen Charlotte Is	1934-1949	14,901	1,381	11:1
Nass River	1930-1949	39,183	20,323	1.9:1
Skeena	1930-1949	51,313	47,933	1.1:1
Grenville-Principe	1930-1949	31,715	13,629	2.3:1
Butedale	1930-1949	59,192	39,430	1.5:1
Bella Bella	1930-1949	17,780	35,780	1:2
Bella Coola	1930-1949	22,538	37,985	1:1.7
Rivers, Smith Inlets	1930-1949	5,008	8,601	1.1.7

TABLE II

RELATIVE ABUNDANCE OF E AND O PINK SALMON IN SOUTHERN B.C., AS INDICATED BY REPORTED ANNUAL ESCAPEMENTS*

Area	*Period*	*Average escapement* (1000's) E	 O	*Ratio* E:O
Quathiaski	1934-1949	213.8	109.5	2:1
Comox	1934-1949	110.4	165.0	1:1.5
Toba Inlet	1934-1949	11.8	117.1	1:9.9
Jervis Inlet	1934-1949	58.1	167.4	1:2.9
Nanaimo	1934-1949	4.0	5.3	1:1.3
Clayoquot	1934-1950	2.8	1.9	1.5:1
Quatsino	1935-1946	167.0	3.8	44:1
Sooke Trap catch*	1938-1949	.474	126.3	1:266

*The Sooke Trap catch is included as being the best available indicator of the ratio between E and O fish travelling to Puget Sound and the Fraser River are transients migrating to other destinations. In particular, the large biennial runs to the Fraser River and Puget Sound pass through and influence the catches of many other fishing areas. For these areas, therefore, comparisons have been made (in Table II) on the basis of reported escapements to the spawning streams. Because of the difficulties involved in making estimates of spawning populations in a vast number of streams of different size and character, these figures are presented only for the purpose of demonstrating the gross features discussed below. Actually, estimates are commonly made as categories, for example "between 2,000 and 5,000", "between 50,000 and 100,000." The figures used have been arrived at by taking the midpoints of the various categories.

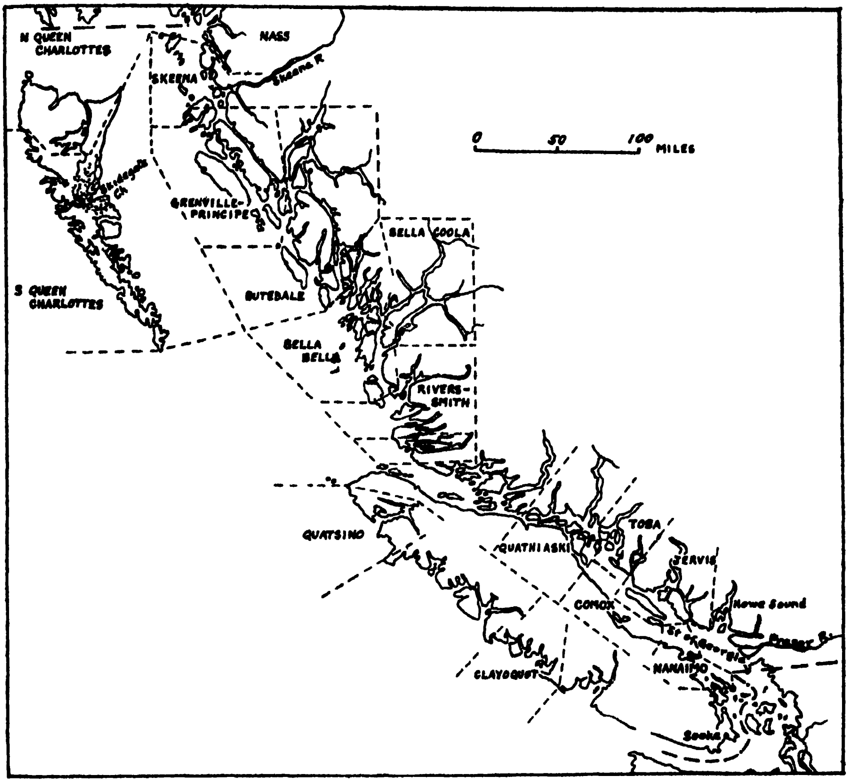


FIGURE 1 —Coastal regions of British Columbia, showing areas referred to in text. Stippling (Queen Charlottes) represents area in which streams show no extreme disparity (see text)

Reviewing the 17 areas represented in Tables I and II, it is at once evident that two of them show an extreme degree of disparity between

E and O fish, the north Queen Charlottes having an overwhelming preponderance of E pinks and the Fraser–Puget Sound runs an equally impressive dominance in the O stocks A third area (Quatsino) shows a pronounced but less extreme disparity favouring the E population. In all other areas the difference, for the periods quoted, is of a much lower order. Although the maintenance for several generations of a two- or three-fold difference in the magnitude of E and O catches sometimes leads in common parlance to the recognition of alternate "off" years and "on" years, the leading position not infrequently changes from one stock to the other (see Figs. 3 and 4). For nearly all of the areas listed in Tables I and II a shorter period of years could be selected which would reverse the preponderance suggested by the ratios given in these tables.

Since each of the areas referred to possesses numerous salmon streams, it would theoretically be quite possible for this general condition of near-equality of the stocks to represent merely the combined output of numerous spawning grounds which, taken individually, might show great disparity between E and O stocks. If E and O fish tended to spawn in different streams, an underlying similarity between the phenomenon observed in the Fraser and Queen Charlotte areas and the situation existing elsewhere could be established.

Observations made by personnel of the Fisheries Research Board and the much more comprehensive surveys of the Department of Fisheries show, however, that there is no such segregation of the breeding places of the two stocks. An examination of Departmental records shows clearly that in general the individual streams reflect closely the ratios shown by the commercial catches in the sea. The writer has seen reports relating to more than 200 pink salmon streams in the "sub-equality" areas of British Columbia, each stream being covered for a period of from 7 to 17 years. In no single instance has he found evidence of a degree of disparity between stocks which is at variance with the general picture obtained from the consideration of large areas. In most cases an overlap is indicated in the abundance of fish belonging to E and O populations.

A few specific examples may be cited from many streams showing a very high order of abundance in both odd and even years. The writer is personally familiar with the streams mentioned.

The Neekas River, in the Milbanke Sound area, has an accessible length of about one mile and an average width of about 20 yards. It supports large runs of both pink and chum (*O. keta*) salmon and the spawning grounds appear to be fully occupied by the presence at any given time of 40,000 salmon. Between 1935 and 1945 estimates of pink salmon alone were of the order of 75,000 or higher in four even years and five odd years.

Evelyn Creek, on Hawkesbury Island, is a very small stream, about one and a half miles long. The flow on September 1, 1945, at the time when the adult fish were beginning to enter, was estimated to be only 6 c.f.s. (this was undoubtedly an unusually low level). Escapements of between 50,000 and 100,000 pink salmon were assigned to this stream in 1942, 1943 and 1945. The assumption of errors up to several hundred per cent would not alter the conclusion that full utilization of available spawning ground was being made by both E and O fish.

The Koeye River, flowing into Fitzhugh Sound, is well known to fishermen and Departmental officers as a large and constant contributor to the catches of the Queen Charlotte Sound area. It is a somewhat larger stream than those previously mentioned but the favourable spawning areas probably do not total more than three miles in length. Both even- and odd-year escapements are commonly estimated as "over 100,000." For recent years of both series this is believed to be a very conservative statement.

While they do not change the general impressions formed from an examination of catch figures, stream reports do, however, enable us to define more precisely the boundaries of the Queen Charlotte Island region which is characterized by extreme disparity. From Table I it would appear that only the northern portion of Graham Island displays this condition in an acute form, the rest of the island group showing only a moderate degree of E dominance. Departmental records make it clear that in fact most of the pink salmon streams of both Graham and Moresby Islands have virtually no runs in the odd years. A few rivers, however, located in a belt which extends on both sides of Skidegate Channel, have good or fair runs in both even and odd years and in fact show almost precisely the same state of affairs as the streams of the opposite (mainland) coast of Hecate Strait (see Fig. 1). Streams which can be named as belonging in this category are: Tlell River, flowing to the east coast of Graham Island; Riley Creek, flowing to the west coast of Graham Island; Copper River, flowing to the east coast of Moresby Island; Deena River, flowing to the north coast of Moresby Island (Skidegate Channel).

As regards the freshwater situation, therefore, most of Graham and Moresby Islands can be designated as a region of extreme E and O disparity, with a small central area which transects this region and which shows a condition characteristic of most other parts of the British Columbia coast.

In the southern mainland region the writer knows of no streams which provide exceptions to the thoroughgoing dominance exhibited by the O fish, although the abundance of these varies considerably in different generations. E fish, however, are not entirely absent, having been reported in small numbers in many of the same watercourses. The disparity is not confined to the Fraser River watershed, being characteristic of streams in the adjacent districts of Puget Sound and Howe Sound. It does not extend to the southern end of Vancouver Island nor to the streams of Jervis Inlet.

The existing conditions with respect to the relative abundance of E and O fish in British Columbia as a whole can be summarized as follows:

1. Throughout the greater part of the range of the species within or near the province, a long-term condition of near-equality exists, with either stock capable of showing greater abundance in certain years or periods.

2. Throughout the periods for which records are available, local conditions of extreme disparity have prevailed in the Queen Charlotte Islands and in the southern mainland area. A less extreme disparity has existed in the Quatsino area of northwest Vancouver Island.

3. The ratios between E and O fish are characteristic of *regions* and are not the exclusive property of individual streams. Even the apparently mixed situation on the Queen Charlotte Islands appears to partake of this regional nature.

2. *In Other Regions*

The total geographic range of the pink salmon, excluding sparse or infrequent occurrences in Oregon and California, extends from the state of Washington northward along the American coast to northern Alaska and the Mackenzie River. On the Asiatic side of the Pacific it ranges southward as far as northern Korea and northern Honshu. Such data as the writer has seen relating to the comparative abundance of E and O fish throughout the greater part of this vast realm are mainly in the form of catch records for very large districts within which there is an obvious possibility of diverse local conditions. Nevertheless, even general information from the much greater territories occupied by pink salmon elsewhere is helpful in assessing the problem of disparity as witnessed in British Columbia.

The following remarks concerning Alaska are based on the annual catch figures supplied by the U.S. Fish and Wildlife Service and the canned packs reported in the *Pacific Fisherman Yearbook.* During the last fifteen or twenty years each of the seven recognized statistical districts of southeast Alaska shows much overlapping in the quantities reported for even and odd years. The total range of variation tends to be of a similar order for both stocks and their relative abundance can be said to be in general accord with the condition which prevails along the greater part of the British Columbia coast. The same may be said of the five districts of central Alaska (comprising the region from Cape Spencer to the Alaska Peninsula) for which pink salmon packs are reported (in Cook Inlet the E fish are considerably more numerous on balance but there is no disparity of the extreme and persistent type to which reference has been made). In western Alaska (Bristol Bay, Yukon, etc.) certain series of figures give the impression of a thoroughgoing disparity favouring the E fish. The record is not consistent in this respect, however, and, since pink salmon catches are always relatively small in this region, it seems unsafe to draw definite conclusions regarding the ratio of the populations.

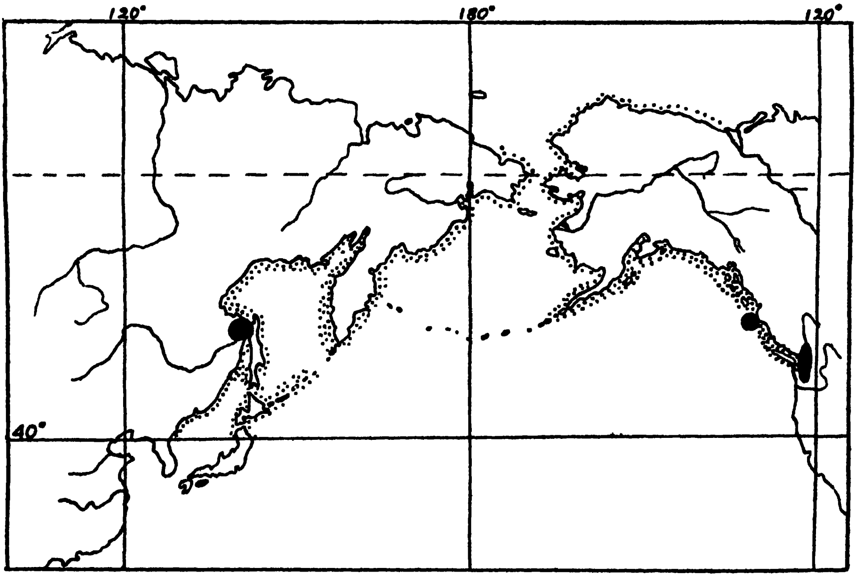


FIGURE 2.—North Pacific region, showing general distribution of pink salmon and (in black) areas of extreme disparity.

On the Asiatic side Pravdin (1940) quotes catch figures for the years 1926 to 1934 inclusive for each of 10 regions of the U.S.S.R. coast. From south to north these are: Primor; Nikolaevsk; Sakhalin; Okhotsk; Gijiginsk; Ichinsk; West Kamchatka; East Kamchatka; Karagin; Olyutoro-Navarin. The figures given show some degree of overlapping between E and O fish for six of these districts (in the two northernmost sections the leading position appears to have swung from the E to the O stock within the period). In the four regions for which no overlap is indicated, the ratio is nevertheless quite low (7.5:1 or less) in two cases. Of the two remaining districts one, Gijiginsk, appears to be a relatively unimportant producer of pink salmon. The ratio obtained from Pravdin's figures is 31:1 in favour of the E stock—or roughly the condition prevailing in the Quatsino Sound area of British Columbia. The other exception is Nikolaevsk, which includes the very important fisheries at the mouth of the great Amur River. Here, the indicated disparity is even more extreme than that of the Fraser River area—but in favour of the E stocks. A further parallel can be seen in that the condition apparently does not extend to Sakhalin Island, the geographic relationship of which to the Amur is rather like that of Vancouver Island to the Fraser. Of especial interest, however, is the information supplied by Berg (1928) that the condition in the Amur district is of recent origin. Berg's figures for pink salmon caught annually near the mouth of the Amur are as follows (in thousands of individuals).

Odd Years		Even Years	
Year	*Number*	*Year*	*Number*
1907	1,478	1908	4,600
1909	2,950	1910	7,701
1911	3,856	1912	7,528
1913	7,469	1914	14,528
1915	994	1916	9,243
1917	557	1918	14,876
1919	420	1920	12,000
1921	200	1922	9,000
1923	18	1924	12,217
1925	0		

Pravdin's later data are given by weight. The following figures represent "thousands of centners" (a rough calculation on the basis of an average weight of 4 lb. per individual fish would give 27.5 fish per centner (= 50 kg.) and indicates that the 1926 catch was down to less than 5,000,000 fish).

Odd Years		Even Years	
Year	*Weight*	*Year*	*Weight*
		1926	171.5
1927	0	1928	116.5
1929	0	1930	73.2
1931	0.3	1932	75.49
1933	0.39	1934	53.4

The implications are that a very moderate preponderance of E fish became exaggerated by a slump which appeared in the offspring of the 1913 run. The decline in O fish continued for the next five generations by which time commercial production was nil. During this time the E fish continued to maintain themselves at a high level. Pravdin's figures for the ensuing nine years indicate that the catches of E fish declined during this period but with little revival of the O stocks.

This brief review of conditions obtaining in other parts of the geographic range of the species tends to confirm the impressions formed from a consideration of British Columbia data alone, namely, that a state of semi-equality of E and O stocks is usual and that extreme disparity, while it occurs in widely separated areas, is local and infrequent.

CHANGES IN ABUNDANCE OF E AND O STOCKS

Figs. 3 and 4 show reported catches for various areas of the northern and central British Columbia coast over a period of approximately 20 years. Although, as stated previously, precise quantitative accuracy is not claimed for these records, the writer is confident that the direction of major changes in abundance is in general disclosed by these graphs.

Certain consistencies are apparent. In all areas except the north Queen Charlottes, where the O runs are practically nonexistent, each stock occupies a leading position at some time during the period shown. While changes in one stock appear to be independent of changes in the other stock, the direction of change in a given year is frequently similar in many areas. Thus, the E catches show a marked decline in all areas in 1932. Other even-year declines are apparent in nearly all areas in 1940 and 1946. Marked gains were registered in all areas south of the Skeena in 1944 and in all areas in 1948. The odd-year fish showed a decline in all areas except the Skeena in 1935 and heavy declines in all areas in 1941 and 1947. A widespread increase was shown in 1933; in 1939 all areas showed gains; in 1943 a very heavy increase occurred in all areas south of the Skeena, and in 1949 there was a substantial improvement everywhere.

These common tendencies suggest strongly that changes in abundance are frequently determined by factors which operate over relatively large regions.

DISCUSSION

Reference is made again to the question how or why two populations with similar requirements and opportunities can continue to inhabit the same area on a totally different scale of numerical abundance.

The view that the smaller population is held down to a low level by the existence of the larger population may be considered first. Certain points which appear to favour this view are:

1. Great disparity in the stocks of pink salmon is characteristic of the Fraser River system, where the "dominance" of certain sockeye stocks has also been conspicuous The disparity in the latter species has

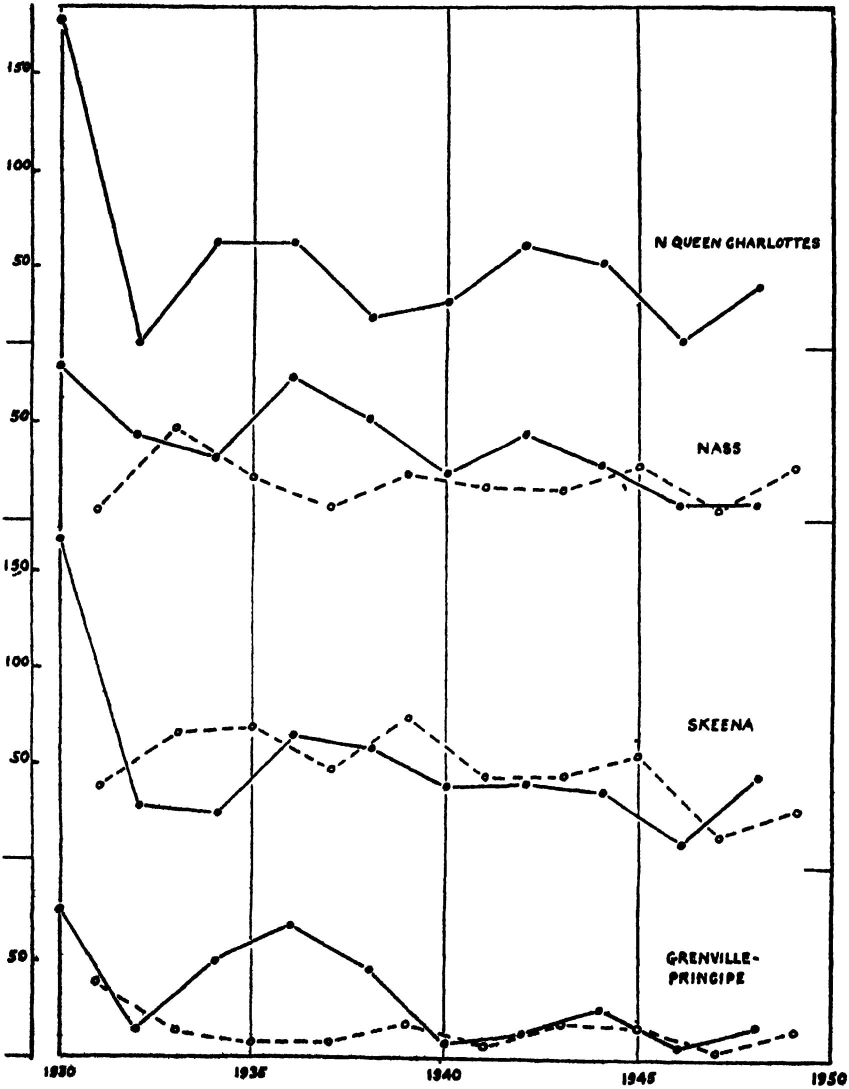


FIGURE 3.—Reported annual catches (thousands of cwt) of pink salmon in areas of northern British Columbia. Solid line—E fish. Broken line—O fish.

been thought to be due to interaction between year-classes (Ricker, 1950) and it seems reasonable to seek a common explanation for the two species.

2. Attempts to establish "off-year" pink salmon runs in a stream possessing an abundant population of the other stock have been unsuccessful (Pritchard, 1938).

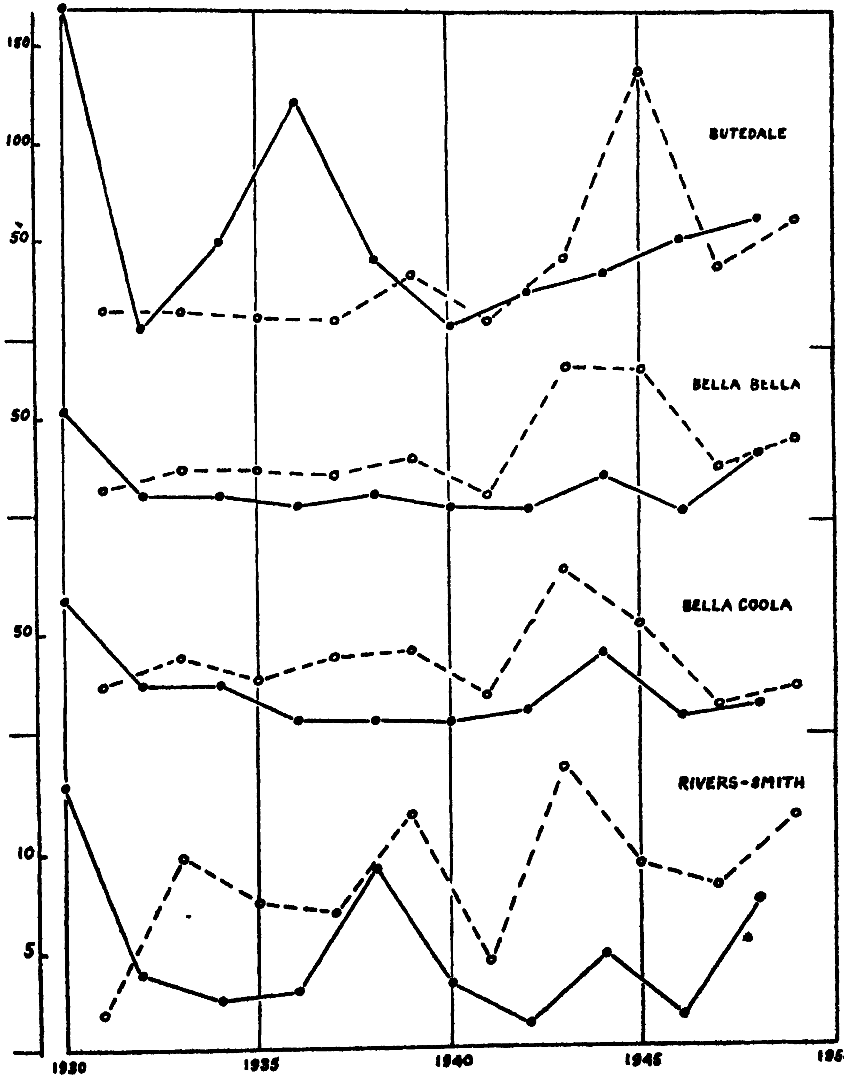


FIGURE 4 —Reported annual catches (thousands of cwt.) of pink salmon in areas of central British Columbia Solid line—E fish. Broken line—O fish.

3. In the only documented instance of the inception of a state of extreme disparity in pink salmon (Amur River, see above), the decline of one population apparently synchronized with an increase in the other population.

Before presenting arguments which oppose this view, it may be pointed out that when two populations continue to exist at their respective levels (as seems, broadly speaking, to be the case in the Fraser system) each is being equally successful in reproducing itself. The smaller population is only being "held down" (relative to the larger one) if it is assumed that the higher population represents a more "natural" state to which the smaller stock should be approximating. The large population could in fact only be "holding down" the small one to its own reproductive rate. If, on the other hand, there are factors which tend to keep populations at or near their existing levels, continued disparity requires only an initial cause to keep it in being. In any case, it would seem that *production* of disparity is a different matter from its maintenance, since the former requires that the survival rates of the stocks be different whereas the latter requires that they be the same—that is, unless one stock is totally absent.

The view that the abundance of one stock inhibits the abundance of the other stock does not seem to be supported by the general geographic evidence already cited. Extreme disparity is exceptional (see Fig. 2). There are many regions, including such centres of abundance as southeast Alaska and central British Columbia, in which both stocks can show very high levels of population size. There is no evidence of a general reciprocal relationship between the size of E and O stocks. It is possible to cite examples which seem directly opposed to such an idea. For instance, the rise of the O stock to an extremely high level in 1943 in the Bella Bella, Bella Coola, and Rivers Inlet areas was followed by a marked increase in the E stocks of these areas in 1944 (Fig. 4). In the previously cited case of the establishment of a strong disparity in the Amur district, the initial decline of the O fish does indeed appear to have synchronized with an increase in the E stock. But if the available catch figures are accepted as indicative of the condition of the runs, the E population then remained nearly constant for a series of generations, whereas the O stock continued to dwindle with each successive life-cycle. The subsequent decline of the E stock in the years 1926 to 1934 seems to have elicited no such marked or rapid revival of the O fish as might be expected if this were the factor which was holding them down.

If, however, it is conceded that the general evidence concerning the independence of the two stocks is insufficient to exclude the possibility of interaction as a cause of continued disparity, it is necessary to consider the periods in the life-cycle when such interaction might occur. In the case of the sockeye an explanation has been sought in terms of intraspecific competition or intraspecific predation during the rather lengthy period of lake residence. Since no such period occurs during the life-history of the pink salmon, no similar hypothesis can be set up for this species. There is in fact no overlapping between stocks at any stage of freshwater existence, and the only possibility of interaction in this medium would seem to lie in one stock rendering the habitat unsuitable in some way for the population of the following year. This possibility can be discounted in view of the very heavy pink salmon populations which do in fact occupy the same stream areas year after year in many parts of the geographic range of the species. Specific examples in British Columbia have been cited previously.

While evidence has been presented elsewhere (Davidson and Hutchinson, 1943; Neave, 1952) that pink salmon abundance tends to be determined by freshwater factors, the possibility that the condition of extreme disparity is maintained by interaction between E and O stocks in the ocean remains to be considered. On general grounds it seems rather unlikely that successive year-classes can establish such exclusive interrelations that they can determine their relative abundance without reference to the vast and varying numbers of other predators or competitors in the unrestricted environment of the sea. Other objections can also be raised.

Underyearling pink salmon, which from their numbers, distribution, and greater abundance in even years undoubtedly include many Fraser River fish, are to be found in the Strait of Georgia and among the San Juan Islands until July and early August (Foskett, 1951; Clemens, 1952). The fish of the other stock by this time have nearly completed their marine feeding period and are on their return journey to fresh water. They are concurrently contributing to the commercial fishery on the southwest and north coasts of Vancouver Island. If the same routes and depths are followed, the outward and inward migrations must be in close relation at some period, but it may be seriously doubted whether there is any prolonged feeding in common areas by different year-classes of fish which migrate extensively during such a relatively short period of marine life.

The view that continued disparity is the result of competition or predation between E and O populations seems to lead to the inference that in areas where both are abundant the stocks are not in contact at the appropriate period. The writer finds it difficult to think that there are a few special areas scattered within the wide range of the species wherein such contacts have been able to produce a continued eclipse of one stock whereas in other areas the abundance of both seems entirely compatible. The difficulty becomes acute in the case of the Queen Charlotte Islands. Here it would have to be supposed that the marine phases of the O fish of a few streams are insulated from a form of predation or competition which imposes a continuous depression on the fish derived from nearby streams on all sides of them.

In seeking an alternative explanation for the occurrence of extreme and persistent disparity, the possibility might be considered that one stock has never been able to establish itself in certain areas. The apparently complete dichotomy of the species into E and O stocks is presumably a relatively recent event. The geographic areas suitable for pink salmon must have changed considerably during postglacial times. Once segregation into the two groups had been effected, colonization would be a separate process in each case and would not necessarily proceed at the same rates in all regions. The Queen Charlotte Islands might well present a difficult area for colonization from the mainland by a species with pronounced homing tendencies. The small area on these islands which is occupied by appreciable numbers of O fish might be interpreted as the bridgehead of an invasion which is now in progress. In the Fraser–Puget Sound area, which lies at or near the southern limit of the present distributional range, it might be argued that the E fish have arrived too recently to have encountered the specially favourable conditions necessary for a great expansion of population.

Such a theory would not account for the recently achieved disparity of the Amur River district. It must therefore be concluded that some and perhaps all cases of extreme disparity have been initiated by a different survival rate between stocks which were formerly on a more equal footing. As already pointed out, this different survival rate could not have persisted in cases where the two stocks are maintaining their relative positions and might indeed have been a very brief phenomenon.

In connection with Figs. 3 and 4, it has been noted that changes in abundance of pink salmon from one generation to the next are regional in their application. This is true also of the occurrence of extreme disparity. The latter may reasonably be thought to have been initiated by factors similar to those which induce the variations commonly seen in pink salmon stocks. These variations have been related to meteorological conditions affecting the freshwater phases of the life-history (Davidson and Hutchinson, 1943; Neave and Wickett, 1949; Neave, 1952). Such conditions can be regional in effect, and in a given year would affect only one stock. Factors which could adversely affect one stock in a regional manner include: droughts; heavy floods; unfavourable water temperatures; exposure and freezing of spawning grounds.

Increased survival is promoted by the maintenance of relatively constant water levels and favourable temperatures during the freshwater period. A mechanism evidently exists for initiating a difference in abundance between the E and O stocks.

Regarding the further development and fixation of disparity, the writer (Neave, 1952) has emphasized elsewhere the existence of factors which tend to confirm and exaggerate changes in population size, as well as factors which tend to promote stability. Attention was especially directed to the probable effect of predators during the migration of salmon fry to the sea, in inflicting relatively heavier losses on smaller populations. When a population exceeds or falls below certain limits of size it is likely to be subject to influences which tend to maintain the direction of change or at least to oppose a return to the former level.

The Amur data suggest that a disaster attending the reproduction of the 1913 spawning stock depressed the 1915 population to a point well below the levels recorded in previous generations. From this point the decline continued in each generation, resulting in commercial extinction in 1925 (of the escapement in that year Berg says "there were only single specimens that ascended small rivers falling into the Amur below Nikolayevsk"). The other stock maintained itself well during this period. It can hardly be supposed that good and bad reproductive seasons alternated regularly for 12 years. We must accordingly conclude that the initial catastrophe brought its own train of subsequent events. Conceivably, disparity could also result from an *increase* of one stock. Given an exceptionally favourable reproductive season, a population might "break through" the existing predator control and attain a relatively permanent high level of abundance. To attain extreme disparity in this way, however, the process would have to start from a relatively low level of the stocks, since limitations of habitat other than predators would certainly stop expansion at some point.

It is not necessary to assume that a major downward change inevitably proceeds to the extreme degree shown in the case of the Amur. Stabilization might occur at some relatively low level at which the young fish begin to be less available to predators and the reproductive efficiency of the population is higher than that of a large population. The evidence indicates that pink salmon populations can be stabilized (relatively speaking) at various levels, at least when in different localities. The Amur case does suggest strongly, however, that between a high status and a low status there may be intermediate levels of abundance which are very difficult to maintain under natural conditions. In cases where disparity has become acute, chances of a small population attaining the status of the large one may be very small.

Extreme, persistent disparity is a rare condition in pink salmon. Apparently the limits within which the populations can change without being definitely committed to a new status are broad enough to permit very considerable fluctuations and are only transcended under highly exceptional and infrequent circumstances.

Those who are interested in the practical problem of promoting large pink salmon runs in "off" years should consider the possibility that a gradual increase of the existing stocks or the planting of a few hundred thousand eggs to start a new population may frequently not be a feasible approach to the problem unless supplementary measures are taken. On the other hand they can derive satisfaction from the observed fact that large as well as small or negligible populations can exist in many waters, and from the evidence that the two states are not reciprocal. Present knowledge suggests that the change from a low to a high status should be made as far as possible in one "jump," or that the factors (believed to be especially the removal of fry-migrants by predators) hindering a more gradual increase should be eliminated.

ACKNOWLEDGMENT

The writer is indebted to the officers of the Department of Fisheries for access to reports and records which he has used freely in developing the views expressed in this paper.

REFERENCES

Alaska fishery and fur seal industries. U.S. Fish & Wildlife Service, Washington, D.C.

BERG, L. S. (1928). The Pacific Research and Fishery Station at Vladivostok. Proc. 3rd Pan-Pac. Sci. Congress, 2239-40.

CLEMENS, W. A. (1952). On the migration of Pacific salmon (*Oncorhynchus*). Trans. Roy. Soc. Canada, 3rd ser., **45** (sec. V): 9-17.

DAVIDSON, F. A. and HUTCHINSON, S. J. (1943). Weather as an index to the abundance of pink salmon. Pac. Fisherman, May 1943.

FOSKETT, D. R. (1951). Young salmon in the Nanaimo area. Prog. Repts. Pac. Coast Stations, **86**:18-19.

NEAVE, F. Principles affecting the size of pink and chum salmon populations in British Columbia. J. Fish. Res. Bd. Canada (in press).

NEAVE, F. and WICKETT, W. P. (1949). Factors affecting the freshwater development of Pacific salmon in British Columbia. MS., Pac. Sci. Congress.

Pacific Fisherman Yearbook. Seattle.

PRAVDIN, I. F. (1940). (Investigations of far eastern salmon.) Bull. Pac. Sci. Inst. Fish. and Oceanog., **18**:1-107.

PRITCHARD, A. L. (1938). Transplantation of pink salmon (*Oncorhynchus gorbuscha*) into Masset Inlet, B.C., in the barren years. J. Fish. Res. Bd. Canada, **4**:141-150.

RICKER, W. E. (1950) Cycle dominance among the Fraser sockeye. Ecology, **31**:6-26.

TRANSACTIONS OF

THE ROYAL SOCIETY
OF CANADA

SECTION V
BIOLOGICAL SCIENCES

THIRD SERIES—VOLUME XLVII—SECTION V
JUNE, 1953

OTTAWA
THE ROYAL SOCIETY OF CANADA
1953

TABLE OF CONTENTS

On Some Fundamental Problems in the Biology of Pacific Salmon. Presidential Address by W. A. CLEMENS, F.R.S.C. . . . 1

The Story of Listeria. Flavelle Medallist's Address by E. G. D. MURRAY, F.R.S.C. 15

The Postglacial Pine Period. By PIERRE DANSEREAU, F.R.S.C. 23

Life's Progressive Orderliness based on the Binary Statistic of Genic Alleles. By A. H. HUTCHINSON, F.R.S.C. 39

The Implications of Recent Advances in Medical Bacteriology. By E. G. D. MURRAY, F.R.S.C. 45

The Use of Plant Material in the Recognition of Northern Organic Terrain Characteristics. By NORMAN W. RADFORTH . . . 53

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVII : SERIES III : JUNE, 1953
SECTION FIVE

PRESIDENTIAL ADDRESS

On Some Fundamental Problems in the Biology of Pacific Salmon

W. A. CLEMENS, F.R.S.C.

MANY investigations have been carried out over the past forty years on the five species of Pacific salmon, genus *Oncorhynchus*, occurring on the North American coast. Many of these studies have been fact-finding in character with the objective of providing information for the management of the fisheries based upon the populations of these species. At the same time, basic biological problems have been given considerable attention and a few of these will be discussed briefly in this paper.

ORIGIN, DISTRIBUTION, AND SPECIATION

The evolution of the salmonoid fishes probably began in the Eocene period and the genera *Salmo* and *Oncorhynchus* were distinct by the Miocene. Apparently the majority of ichthyologists today accept the separation of the genera *Salmo* and *Oncorhynchus*. Regan (1914) believed that the two genera were not separable but Tchernavin (1938) presented evidence supporting their distinctness.

Of the evolution of the species of *Oncorhynchus* one can only speculate. Tchernavin (1939) indicates a freshwater origin for the genus *Salmo*. If this theory is accepted, the genus *Oncorhynchus* is undoubtedly also of freshwater origin and species formation may be visualized through isolation in river systems with subsequent acquisition of sea-going habits and spread to numerous river systems. Retention of distinct spawning times or locations would prevent extensive hybridization.

On the coast of North America five species of *Oncorhynchus* are recognized, namely: ***tchawytscha*** (spring, king, chinook), ***kisutch*** (coho, silver), ***gorbuscha*** (pink, humpback), ***keta*** (chum, dog), and ***nerka*** (sockeye, red, blueback). On the coast of Asia several additional species have been described, some of which are probably of subspecific rank. The following remarks are confined to the North American species.

The five species of *Oncornynchus* mentioned above are separable on the basis of many morphological characters such as numbers of scales in rows along the sides of the bodies, numbers of gill rakers, branchiostegals, fin rays, pyloric caeca, coloration, and of differences in behaviours and life histories (Clemens, 1935; Foerster and Pritchard, 1935; Milne, 1948). These specific differences may have been established through isolation of populations of the original *Oncorhynchus* stock in early Pleistocene and subsequent years. That the separation of the species may have been of comparatively recent date is indicated by the fact that cross-breeding is possible, as shown by the cross-fertilization experiments carried out by Foerster (1935). His results show that of twenty possible crosses five were excellent or good, four were moderately good, and eleven were poor or failures. Only between sockeye and chum salmon were the reciprocal crosses good as shown by the following·

Male	*Female*	
sockeye	chum	good
chum	sockeye	good
sockeye	spring	poor
spring	sockeye	excellent
chum	pink	excellent
pink	chum	poor
pink	spring	excellent
spring	pink	moderate

Hourston (1949), in a preliminary study of the serological relationships among some of the species, finds a fairly close similarity among coho, spring, and sockeye and a significant difference between the chum and the above-mentioned three species.

Brett (1952), in a study of temperature tolerance among young Pacific salmon, determined that the upper lethal temperatures were: spring, 25.1°C.; coho, 25.0°C.; sockeye 24.4°C.; pink, 23.9°C.; and chum, 23.8°C.

On the basis of morphological, physiological, life-history, and behaviour studies to date, it appears that spring and coho salmon are related on the one hand, pink and chum on the other, and that sockeye occupy a position more or less intermediate between the two pairs.

A promising line of investigation on the origin and relations of the species is indicated by the study of chromosome numbers in Salmonidae by Svärdson (1945). He finds that the diploid chromosome number of the Atlantic salmon, *Salmo salar*, is 60 and of the brown trout, *S. trutta*, 80. He postulates that the basic number in the Salmonidae must have been 10 and the present day salmonoids must accordingly be high-polyploids. His conclusions are as follows:

Salmo salar, Atlantic salmon	6 ×	= 60
S. trutta, brown trout	8 ×	= 80
S. alpinus, Arctic char	8 ×	= 80
S. fontinalis, speckled char	8 × + 4	= 84
Coregonus lavaretus, whitefish	8 ×	= 80
C. albula, whitefish	8 ×	= 80
Thymallus thymallus, grayling	10 × + 2	= 102

Makino (1937) stated that the diploid chromosome number for the chum salmon, *O. keta*, was 74. Svärdson believes this to be of the correct order of magnitude but not quite exact. Mr. James G. Robertson, a graduate student in the Department of Zoology, University of British Columbia, obtained a count of 38 chromosomes for this species but again this is probably not accurate and it is uncertain whether this is a haploid or a diploid number.

It is not improbable that the Pacific salmon are polyploids. If so, at least some of the species may have originated by polyploidy rather than by micromutations. On this basis it may be predicted that the pink salmon will be found to have the smallest chromosome number as it is the smallest of the five species, averaging but four pounds in weight. On the other hand, the spring salmon will be expected to have the largest chromosome number as it is the largest in size and may reach a weight of over 100 pounds.

On the basis of the conception of polyploidy in *Oncorhynchus*, the results of Foerster's cross-fertilization experiments and Hourston's serological studies are not necessarily in conflict in regard to the relationship between sockeye and chum salmon. The crosses between these two species were very successful and this may have been the result of compatibility in chromosome numbers while the difference in the nature of the serum proteins may not have been critical.

It may be conceived that a number of species of *Oncorhynchus* evolved early in the Pleistocene and became distributed along the west coast of North America from Alaska to California. What may have been the distribution of the various species during most of this period is difficult to visualize. The only fossil record of *Oncorhynchus* of which I am aware is that given by Hay (1929) for Pleistocene beds in Oregon. However, from the time of the last glacial period onward, a reasonably clear picture of events may be developed. At the height of the last glacial period, some 15,000 years ago, there could have been no streams from the southern area of Alaska to the northern section of the State of Washington. The Yukon Valley was not glaciated and it is possible that some species of Pacific salmon were able to spawn in, and the young to survive in, the Yukon River. To the south, the Columbia and other rivers at least as far south as the Sacramento were available and undoubtedly were utilized. If northern and southern populations existed, they probably remained isolated as distinct stocks for many thousands of years. As the glacial ice retreated, innumerable streams came into existence in the region between the Yukon and Columbia rivers and gradually became occupied by the various species. Today the spring salmon has a distributional range from the Sacramento to the Yukon River.

There can be no doubt of the survival of the salmon in the streams to the south and of their dispersal northward, based on knowledge of existing ocean currents and of salmon movements. Survival in the Yukon is uncertain although seemingly possible. Whether dispersal took place southward is also uncertain. Knowledge of ocean currents in the northern area is relatively meagre as is knowledge of the movements of the far northern salmon.

If intermingling of the far northern and the far southern stocks did not occur, distinct racial characters may have developed. A critical study of populations from the southern to'the northern limits of distribution might well reveal considerable differences, even in subspecific rank.

Because of the strong homing tendency among some of the species, gene flow throughout their ranges is undoubtedly limited. For the sockeye salmon which exhibit the homing tendency so strongly, there may well be a tendency for racial differences to be superceded by subspecific differences among the populations of some of the widely differing river systems. This may be true even within major river systems because of their almost invariable return to specific streams. At the same time it may be expected that closely similar streams will tend to have closely similar sockeye since the environments will steadily select any genetic variations in the same direction. The unravelling of this taxonomic complex of species with their possible subspecies, races, and ecads is an imposing task. While the problem is of scientific interest, it has an important relation to attempts to establish or re-establish populations. If over a period of some 15,000 years the various populations in the Fraser River system have become adjusted to specific environmental conditions, it may be impossible to transfer successfully eggs or fry between quite diverse environments. For example, the Cultus Lake sockeye are of small size, the average egg production per female is 4,130, the fish travel only 100 miles from the sea to the spawning ground at an elevation of 190 feet above sea level and spawn for the most part on the gravel beaches at the head of the lake. On the other hand the Chilko Lake sockeye, which are approximately the same size, have an average egg production per female of only 2,700, the fish travel 400 miles to the spawning ground at an elevation of 3,900 feet and spawn in the Chilcotin River below the lake. The young, upon emergence from the gravel, are carried downstream to an area of slow water and some weeks later ascend the river and enter Chilko Lake (Broadhead, 1949). Such differences may have a genetic basis, but they may be only manifestations of a plastic or variable organization which has rather wide ranges of physiological activity and behaviour. It would be well to know the nature of the material being dealt with in fish management procedures.

MIGRATION

The migrations of Pacific salmon have received attention over a long period of years. From 1919 onward a large body of knowledge of the movements of salmon in the ocean and to their spawning areas has been accumulated by means of tagging of sea-living fish and of clipping the fins of seaward migrants. However, it is only in very recent years that the fundamental factors involved in the phenomena of seaward and return migrations have become evident. While the investigations of Dr. A. G. Huntsman in Canada and of Dr. M. Fontaine and associates in France have contributed much to an understanding of the migrations of both anadromous and catadromous fishes, it is the studies of Dr. W. S. Hoar of the University of British Columbia that have provided information specific to the seaward migrations of Pacific salmon. The following account of the behaviour of the juveniles is based upon the publications of Hoar (1951, 1952, 1953).

Sockeye salmon spawn usually in streams tributary to lakes. The fry upon emerging from the gravel are negatively phototactic and remain during the day at the bottom of the streams, where the light is of low intensity. They rise in the water when the light intensity falls, are displaced downstream by the current, and so eventually reach the lake. In some instances spawning takes place below the outlet of a lake and the young fish are carried downstream until they reach a relatively quiet body of water. There they feed and grow, apparently become positively phototactic, and presently head into the current along the shores of the stream and enter the lake

Chum salmon spawn for the most part in the lower sections of coastal streams. The young emerging from the gravel swim vigorously into the current and tend to hold position. They prefer relatively bright light and when the light intensity falls they are displaced downstream and in due course reach the ocean.

The coho young, in contrast to chum salmon, do not go to sea immediately after emerging from the gravel but remain in the stream for a year. They are positively phototactic, but occupy territory along the margins of the stream and so escape displacement. In the following spring, radical changes take place in the behaviour of the yearlings as they transform from parr to smolt. These changes undoubtedly are associated with physiological changes. The fish lose their territorial behaviour and become negatively phototactic. When the light becomes weak, they rise in the water and so are displaced downstream.

The young sockeye enter the lake as fry and remain there until the following spring. As the water temperatures rise, the fish become very active and form into rapidly moving schools near the surface. They show a preference for currents and apparently move with the current as the temperature rises. As Hoar (1953) states, "osmotic stress, thyroid activity and increased rate of growth with extra demands for food are factors which may initiate restless appetitive behaviour and increase the general activity of the fish." Eventually they come under the influence of the outflowing water from the lake and are displaced seaward.

Several exceptions to the above-described cycle of events leading to seaward migration occur. Coho salmon may occasionally mature in resh water. Sockeye salmon frequently remain two years in a lake, and in northern waters may even remain three or four years before going to sea. Probably some of these delays in seaward migrations are the result of retarded development in cold water. Some may be more or less accidental. Foerster (1937) found at Cultus Lake that seaward migration ceased when the surface water temperature went above 15°C. approximately. Evidently the fish retreat from the warm surface water, called a "temperature blanket" by Ward (1932), and so fail to come under the influence of the outgoing current.

Some sockeye individuals, mostly males, remain in fresh water and mature at two, three, or four years of age. These have been designated "residuals" by Ricker (1938).

Finally there are populations of sockeye which are entirely freshwater inhabitants. These are known as "land-locked" or "lake-locked" sockeye or "kokanees." Superficially at least, they are indistinguishable morphologically from sea-run sockeye but a critical study now being carried out by Mr. E. H. Vernon at the University of British Columbia may reveal distinctive characteristics.

Ricker (1940) has postulated that the kokanee has arisen from the anadromous stock through genetic changes. If such be the case, it is evident that the genetic changes must have involved the endocrine system, since the behaviour pattern has been so altered that seaward migration no longer takes place. Segregation would have been established by kokanee spawning at times and in places distinct from the anadromous population. Systematists have accepted the idea of genotypic differences for some time in that they have recognized the kokanee as a subspecies, *O. nerka kennerlyi.*

Another possibility suggests itself, namely, that the kokanee exhibit reverse mutations and so represent, or are a near approach to, the very early freshwater stock of sockeye before the species became anadromous through genetic changes. In this case the sea-run fish are the original mutants.

However, it is possible that no significant genetic features are involved and that the differences between the anadromous, residual, and kokanee stocks are merely variants over a possible range from complete freshwater existence to complete anadronomy. The degree to which any portion of the range is developed may be associated with lake conditions, possibly largely with water temperatures. Some support to this idea is provided by the facts:

1. Anadromous, residual, and kokanee populations may all occupy a given lake.
2. The progeny of anadromous sockeye can be readily retained in fresh water. Ward (1932) described the occurrence of large numbers of sockeye maturing in Lake Shannon following the construction of a high dam on Baker River.
3. It is possible for kokanee to go to sea and mature there. Foerster (1947) marked and released a large number of kokanee yearlings below Cultus Lake and of these seventeen were subsequently taken as five-year-old fish in the commercial fishery.
4. Residuals and kokanee either do not occur in cold water lakes or occur only in very small numbers, so that their development may be associated with lake temperatures.

It would appear that a somewhat similar situation occurs with respect to the steelhead trout (*Salmo gairdneri*) and its freshwater forms, including the Kamloops trout and certain coastal stream populations.

Returning to the problems of migration, it is apparent that behaviour patterns associated with physiological and environmental conditions and changes account for the seaward movements of young Pacific salmon. The details of these interacting factors remain to be investigated.

Salmon juveniles entering the open sea will be carried northwestward for a considerable period by the shoreward northwest currents. Their subsequent movements are possibly wanderings influenced by such ocean conditions as currents, temperatures, and food conditions.

As far as is known, the young fish remain over the continental shelf in the region between the northwest shore and the offshore southeast current. The early period of life in the ocean is one of vigorous feeding, fast growth, and accumulation of fat. In the second, third, or fourth summer, depending upon the species of salmon and the life history, it would seem that a series of endocrine activities on the part of the pituitary, thyroid, and gonads is initiated. At the same time, changes in the metabolic and osmoregulatory mechanisms take place. As a result of these profound physiological changes it is probable that the salmon exhibit an orthokinetic response whereby they tend to aggregate in water of lower salinity. At the same time they become positively rheotactic and swim into the current. These reactions will take them surfaceward, shoreward, and riverward and eventually upstream (Clemens, 1951).

The factors involved in the return to the home stream are not clearly understood but the investigations of Hasler and Wisby (1951) indicate that olfaction may be very significant, possibly on the basis of juvenile imprinting or conditioning.

The salmon proceed upstream until certain "sign stimuli" or "releasers" become effective and spawning takes place. The investigators in the field of ethology have provided a great deal of knowledge concerning the intimate dynamic relationships which exist between the organism and its environment (Tinbergen, 1951; Koch, 1942; Fabricius, 1950). The need for detailed knowledge concerning the behaviours and reactions of the species of salmon is becoming increasingly evident as stream and lake conditions are being changed through man's activities and as efforts are being made to maintain the salmon populations.

Information along the lines discussed in this section also has an important relation to transfers of salmon. Many attempts have been made to establish species of Pacific salmon in regions far removed from their native distributional area in the North Pacific. Perhaps the best summary is that of Davidson and Hutchinson (1938) who list the distributions of eggs and young of four species to the east coast of North America, to South America, to Europe, and to Australia and New Zealand. They point out that the only successful or temporarily successful transfers have been within the water temperature and salinity ranges of the native area, namely 3° to 18°C. approximately and a salinity probably not exceeding 34 parts per 1000. The only truly successful transfer has been that of the spring salmon to the South Island of New Zealand. It may be that the coho or sockeye salmon has been established in southern Chile; Davidson and Hutchinson so record, but no confirmation has been obtained in recent years. Introductions of spring salmon into the Credit River, Ontario, and the St. John River, New Brunswick, and of pink salmon into streams of the State of Maine resulted in returns over several years but the stocks have now disappeared.

From what is now known of the rather intricate relations between the life cycles of the salmon and the environmental cycles, it is not surprising that failures in transfers have occurred. It seems clear that in any future considered attempt to introduce a species into a distant region many things should be taken into account--especially the coastal current systems, but also the ocean temperature and salinity ranges on the one hand and the behaviour patterns of the young of the species and freshwater environmental conditions on the other. Another feature will be mentioned later.

METABOLISM

As indicated in the previous section, considerable changes take place in the metabolic activities of the salmon during the life cycle. Yearling sockeye in the early spring become very active and feed vigorously in rising water temperatures and in association with increased thyroid activity. In due course they reach the outlet of the lake and are displaced downstream to the ocean. During sea residence the fish feed vigorously on marine zooplankton, chiefly Crustacea, grow rapidly, and lay up fat reserves. With the approach of sexual maturity, feeding diminishes and finally ceases as the fish approach fresh water. The body reserves supply materials for the maturing of the gonads and for energy in swimming against the river current and for spawning activities. For some sockeye salmon of the Fraser River the stores must last for a month of vigorous swimming over a distance up to 700 miles at an average rate of about 27 miles per day, then for redd construction by the female and belligerent activity on the part of the male, and finally for the spawning act. Death ensues in the course of several days. Great differences exist among populations of sockeye in the distances travelled. For example, those proceeding to Cultus Lake go 100 miles and to an elevation of 190 feet. Those to Stuart Lake go approximately 700 miles and to an elevation of 2300 feet. Similar differences occur among the populations of the other species.

Most of our present knowledge of the metabolism of Pacific salmon is the result of the investigations on the spring salmon over a period of some twenty years by Greene (1926). Additional fundamental information has been provided by Lovern (1934) and Fontaine (1951) on the metabolism of the Atlantic salmon. (Citation of all the papers of the above-mentioned three authors is considered unnecessary here.) Greene found that an adult spring salmon during its river migration lost 589 grams of protein and 1177 grams of fat from the muscles. During the migration period the ovaries may increase in weight by as much as 2000 grams. It is not pertinent here to review all the data which may have a bearing on the metabolism of salmon in relation to migration, but consideration of the metabolism is pertinent in relation to delays in migration and to the additional energy demands made by the imposition of fish-ways at high dams in the migration route. Excessive energy demands may result in the complete expenditure of the reserves before the fish are able to reach the spawning grounds or in the arrival of the salmon in such an exhausted state that spawning may be impaired or impossible. Knowledge of the metabolism is also important in relation to projects involving transplantations whether within river systems or to distant geographical areas.

It should be possible to determine the average potential energy of a fish of a given population and to calculate the rate of expenditure of the energy reserves in the stream at known' temperatures and velocities. With this basic datum it should be possible to calculate the amount of surplus reserves available for delays or for mounting fish-ways.

The primary need is to recognize that the adult migrating salmon is a balanced mechanism adjusted to operate within a relatively stable cycle of environmental conditions and that changes interposed in the usual environmental sequence may disrupt the functioning of the animal mechanism.

Population Dynamics

By population dynamics is meant those fluctuating or cyclic changes which take place in the numbers of individuals constituting a population. Fishes which have more or less complex life histories, involving extensive movements between fresh and salt water and delicate interrelations with the environment, may be expected to have considerable fluctuations in numbers. This is the case among Pacific salmon, for sometimes a large influx of adults to a spawning area is followed by a small influx in the succeeding generation; conversely, a relatively small spawning population may produce a large return of adults of the next generation. The most critical analysis of fluctuating changes in abundance has been made by Neave (1952, 1953) who makes certain modifications of ideas expressed by Solomon (1949). Only the freshwater portion of the life histories of pink and chum salmon are dealt with, for it is generally believed that fluctuations in abundance are predominantly of freshwater origin. Reference will be made briefly to the former species only.

Pink salmon mature invariably at two years of age. Therefore in any stream the two year-classes are completely distinct. No individuals of one year-class can breed with those of the other and there exists the phenomenon of two populations of a species utilizing a stream for reproduction but remaining as distinct as though occupying two completely isolated streams. The opportunity is presented therefore of comparing two populations within a very circumscribed environment. Neave shows that two populations frequenting a common stream fluctuate independently in numbers and that there is no evidence to suggest that the population of one year has any effect on that of the following. He states that population levels and changes are determined by three types of mortality. One is *compensatory*, in which mortalities become heavier as the population density increases or become lighter as the population density decreases. For example, the crowding of fish on the spawning beds will increase mortalities whereas the occurrence of fewer fish will decrease mortalities. The tendency thus should be for populations to "level off" and become stabilized, but this seldom if ever happens. Another type of mortality usually acts to prevent increase. In this type, known as *depensatory*, mortalities become relatively heavier as populations decrease in density. For example, predators in the stream existing at a fairly constant level of abundance will consume a larger percentage of fry from a small than from a large salmon population. Finally there is another mortality, *extrapensatory*, which is independent of population density. For example, a severe flood will affect a population irrespective of the density of the eggs in a stream bed. Extremely favourable water conditions may act in the opposite direction.

It appears, therefore, that extrapensatory mortalities tend to result in pronounced fluctuations in population size; that once a population is depressed it may be held there by depensatory mortalities until released by a change in extrapensatory mortality or until an important depensatory factor is eliminated. Such an analysis and classification of factors represents a fundamental approach to the problem of accounting for and measuring the significance of the various elements in a complex situation. Once the problem is clarified, it is possible to proceed with a logical series of investigations, the results from which may then be properly evaluated in the over-all picture.

Such a classification of ideas might well be used in studies of the fluctuations in abundance of all the species of salmon. In the case of the sockeye the problem is somewhat more difficult for, in addition to the corresponding incubation and fry migration periods, there is a year of lake residence followed by a more or less lengthy migration from lake to sea. Furthermore, there is the phenomenon of year-class dominance which exists among certain sockeye salmon populations but which may be merely a special phase of fluctuating abundance.

It is encouraging that increasing attention is being given to the discovery of biological principles in the biology of the Pacific salmon because the management problems in connection with the fisheries are of considerable complexity and their solutions require a broad background of knowledge. The genus *Oncorhynchus* constitutes a unique group for researches, the results from which will not only provide important contributions to knowledge but also lead to significant applications in the conservation of a very valuable fishery.

REFERENCES

Brett, J. R. (1952). Temperature tolerance in young Pacific salmon, genus *Oncorhynchus*. J. Fish Res. Bd Canada, **9**: 265–323

Broadhead, G. (1949). A study of the fecundity of the sockeye salmon (*Oncorhynchus nerka*) in the Chilko spawning area and a comparison with the sockeye salmon of the Cultus Lake spawning area B.A Thesis, University of British Columbia.

Clemens, W. A. (1935). The Pacific salmon in British Columbia waters. Rept. Comm. Fish. B.C., 103–5.

——— (1951). On the migration of Pacific salmon (*Oncorhynchus*). Trans. Roy. Soc. Can., 3rd ser., **45** (sec V): 9–17

Davidson, F. A. and Hutchinson, S J. (1938). The geographic distribution and environmental limitations of the Pacific salmon (genus *Oncorhynchus*). Bull. U.S. Bur. Fish., **48**: 667–92

Fabricius, E. (1950). Heterogeneous stimulus summation in the release of spawning activities. Fish. Inst. Freshwater Res., Drottningholm, Rept., **31**: 57–99.

Foerster, R. E. and Pritchard, A L. (1935). A study of the variation in certain meristic characters in the genus *Oncorhynchus* in British Columbia. Trans. Roy. Soc. Can., 3rd ser., **29** (sec. V): 85–95.

Foerster, R. E. (1935). Inter-specific cross-breeding of Pacific salmon. Trans. Roy. Soc. Can., 3rd ser., **29** (sec. V): 21–33.

——— (1947). Experiment to develop sea-run from land-locked sockeye salmon (*Oncorhynchus nerka kennerlyi*). J. Fish. Res. Bd. Canada, **7**: 88–93.

——— (1937). The relation of temperature to the seaward migration of young salmon (*Oncorhynchus nerka*). J. Biol. Bd. Canada, **3**: 421–38.

Fontaine, M. (1951). Facteurs externes et internes régissant les migrations des poissons. Colloque Internat. Ecol., Ann. Biol., **27**: 569–80.

Greene, C. W. (1926). The physiology of the spawning migration. Phys. Rev., **6**: 201–41.

HASLER, A. D. and WISBY, W. J. (1951). Discrimination of stream odors by fishes and its relation to parent stream behaviour. Amer. Nat., **85**: 223–38.

HAY, O. P. (1929). Second bibliography and catalogue of the fossil vertebrata of North America. Carnegie Inst. Wash., Pub. no. 390, **1**: 752.

HOAR, W. S. (1951). The behaviour of chum, pink and coho salmon in relation to their seaward migration. J. Fish. Res. Bd. Canada, **8**: 241–63.

——— (1951). Hormones in fish. Univ. Toronto Studies, Biol. ser., no. 59, Pub. Ont. Fish. Res. Lab., no 71: 1–51.

——— (1952). Thyroid function in some anadromous and landlocked teleosts. Trans. Roy. Soc. Can., ser. 3, **46** (sec. V): 39–53.

· (1953). The behaviour of juvenile Pacific salmon with particular reference to the sockeye (*Oncorhynchus nerka*) J. Fish. Res. Bd. Canada, **11** (in press).

HOURSTON, W. R. (1949). The serological relationships of some Pacific coast Salmonoid fishes. M.A. thesis, University of British Columbia.

KOCH, H. J. (1942). Cause physiologique possible des migrations des animaux aquatiques. Ann. Soc. Roy. Zool. Belg., **73**: 57–62.

LOVERN, J. A (1934). Fat metabolism in fishes. IV. Mobilisation of depot fat in the salmon. Biochem. J., **28**: 1955–60.

MACKINNON, D. and HOAR, W. S (1953). Responses of coho and chum salmon fry to current. J. Fish. Res. Bd. Canada, **10**: 523–38.

MAKINO, S. (1937). Notes on the chromosomes of some teleost fishes. Zool. Mag., **49**: 75–6.

MILNE, D. J. (1948). The growth, morphology and relationship of the species of Pacific salmon and steelhead trout. Thesis, McGill University.

NEAVE, FERRIS (1952). "Even-year" and "odd-year" pink salmon populations. Trans. Roy. Soc. Can., 3rd ser , **46** (sec. V): 55–70.

——— (1953). Principles affecting the size of pink and chum salmon populations in British Columbia. J. Fish Res. Bd Can., **9**: 450–91.

REGAN, C. T. (1914). Systematic arrangement of the fishes of the family Salmonidae. Ann. Mag. Nat. Hist., ser. 8, **13**: 405–8.

RICKER, WILLIAM E. (1938). "Residual" and kokanee salmon in Cultús Lake. J. Fish. Res. Bd. Canada, **4**: 192–218.

——— (1940). On the origin of kokanee, a fresh-water type of sockeye salmon. Trans Roy. Soc. Can., 3rd ser., **34** (sec. V): 121–35.

SVARDSON, GUNNAR (1945). Chromosome studies on Salmonidae. Kungl. Lantbruksstyrelsen Medd. St. undersokn.-o. forsoksanst. f. sotvattensfisket Nr. 23 (Rept. Swedish State Inst. Fresh-water Fishery Res. Drottningholm) Stockholm, 1–151.

SOLOMON, M. E. (1949). The natural control of animal populations. J. Animal Ecol., **1** : 1–35.

TCHERNAVIN, B. (1938). Notes on the chondrocranium and branchial skeleton of *Salmo*. Proc. Zool. Soc. London, ser. B, **108** (2): 347–64.

——— (1939). The origin of salmon: Is its ancestry marine or freshwater? Salmon and Trout Mag., no. 95: 120–40.

TINBERGEN, N. (1951). The study of instinct. Oxford University Press.

WARD, H. B. (1932). The origin of the landlocked habit in salmon. Proc. Nat. Acad. Sci., **18** : 569–80.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVII : SERIES III : JUNE, 1953
SECTION FIVE

FLAVELLE MEDALLIST'S ADDRESS

The Story of Listeria

E. G. D. MURRAY, F.R.S.C.

IN May, 1924, six cases of sudden death occurred in young rabbits in the animal breeding establishment of the Department of Pathology at Cambridge, with many more during the succeeding fifteen months. The interesting characters presented by the disease and the increasing mortality invited investigation. Although there seemed to be every reason to suppose the disease was an infection, the recognition of the agent proved troublesome, but by August a small gram-positive motile rod was isolated, which reproduced the disease characteristically in rabbits, guinea-pigs, and mice. By far the most striking character of this disease was the production of a large-mononuclear leucocytosis, and, as no similar micro-organism could be found in the literature, we called this new microbe *Bacterium monocytogenes*, in 1926.

Shortly before the detail of all this was published, Harvey Pirie discovered the same bacterium during August, 1925, in four wild gerbilles (*Tatera lobengulae*) in the course of his investigations of plague in South Africa, and, struck by the marked necrosis of the liver, he called the microbe *Listerella hepatolytica*, in 1927. It is a misfortune that the Rules of Nomenclature imposed on Pirie a need to change the generic name to *Listeria* in 1940, as the name *Listerella* had been used for a mycetozoon; and even the legitimacy of this later selection is in some doubt. Nevertheless, if I may be allowed the rhyme of the old name with that of a famous nursery heroine, I would like to tell *The Story of Listerella*, by analogy to the old fairy-tale, in which the unconsidered little girl became a personage of high importance.

I shall avoid entangling the main thread of the story with a recital of bacteriological, immunological, pathological, and clinical details, all of which are available to specialists in a quite profuse literature, because my present purpose is to bring out features of more general interest in the hope of intriguing the imagination by the wide biological rather than the narrow specialized aspects of the subject. I shall also disregard the chronological sequence, in order to group together related interests with some measure of continuity, and integrate them with as much perspection as is possible.

A general interest in this disease is provoked by its wide geographic distribution and the surprising variety of host species in which it has been found. Serious epizootics in domestic animals give it a measure of economic importance and a number of sporadic human cases add to its significance. That it may yet prove to be one of the causes of the syndrome known as Infectious Mononucleosis in man is a controversial matter. In general it appears to have a low morbidity rate with a high mortality and its epidemiology and transmission are unknown, although a seasonal incidence and some relation to feeding and general hygiene have been noticed in domestic animals. The rarity of positive blood cultures is taken as an indication that transmission by blood-sucking arthropod vectors is unlikely

So far as I know, *Listeria monocytogenes* has been isolated from natural disease in eighteen species of wild or domestic mammals and birds; in addition some others have been infected experimentally, and there are also the human cases reported from time to time. There is no reason to suppose the list is yet complete; it involves birds, rodents, ungulates, carnivores, and primates. Instances in these species have been found in nineteen different countries spread over every continent, and quite recently the interest in the disease seems to be increasing.

The form of the disease varies somewhat with the kind of host; it is predominantly a meningo-encephalitis in cattle, sheep, goats, and swine, and in some of these there is thought to be a preceding rhinitis; a metritis, with abortion, occurs in sheep, goats, swine, and cattle and the micro-organism has been found in the genital tract of rabbits, guinea-pigs, horses, cattle, and humans, in birds there is a marked myocarditis, which is also frequent in guinea-pigs; the disease is distemper-like in the fox; there is a conjunctivo-keratitis, which is quite peculiar, in rabbits, guinea-pigs, and horses; in many of these and in other animals it is a systemic infection marked by a necrotic hepatitis, but in the ferret it is a mild disease, almost a carrier state. In man many cases have been meningitis, some septicaemic; and a few cases in which *Listeria* has been isolated were clinically typical infectious mononucleosis. Quite evidently these various predispositions to host-selective lesions offer a special opportunity for the study of comparative pathology, since there is no essential difference in the strains of *Listeria monocytogenes* isolated from the varied conditions and different hosts. It has been suggested that this form of meningo-encephalitis might afford an experimental means of studying the blood-brain barrier, especially since a marked angeitis of the smaller vessels of the brain has been observed.

A special form of the human disease has been described in Germany and called "granulomatosis infantiseptica" and it is interesting that one of the two human cases so far found in Canada conforms to this type. At least twenty-seven fatalities in newborn infants were found in Halle; these cases were generalized infections with extensive necrosis of the liver and *Listeria monocytogenes* was isolated from various organs. An identical *Listeria* was isolated from the vagina of the mothers, who remained well. This is interesting because of the metritis and abortion described in various kinds of domestic animals and the finding of *Listeria* in the genital tract of others, and, in addition, because in the original epizootic in the Cambridge rabbits the mortality was in the newly weaned baby rabbits, for the most part, and their mothers remained well. Even though sporadic cases occur in adult animals and human beings, these observations indicate a situation of peculiar interest and raise the general problem of age susceptibility to specific infections.

A finding with wider implications concerns twenty-four lemmings brought by Dr. T. H Manning from Chesterfield and Morse Island in the Canadian Arctic to Ottawa, in the fall of 1949. Within a week of their arrival they were found to be dying of *Listeria monocytogenes* infection. It cannot be positively affirmed that they did not become infected on the way from Churchill to Ottawa, but it is interesting that another lot brought to Toronto more recently have the same disease. In no epizootic has listeriosis proved an exterminating disease; it has a relatively low incidence with a partiality for the immature and in the original Cambridge finding we noticed a definite relation to feeding in both incidence and recovery. The observations published on the Alaska 1949 cyclical mortality of lemmings describe tundra vegetation denuded by the lemmings without tangible indication of starvation. Rausch who made the Alaska report found "Dead lemmings were much in evidence, scattered over the bare tundra and surrounding snow." The greatest mortality was in juveniles. Convulsive movements in the dying were noticed and "there were no grossly visible lesions such as might be expected from bacterial infections," but the lemmings brought south and proved to have listeriosis frequently did not show gross lesions either. In the Alaska mortality the rapacity of owls, jaegers, foxes, and dogs for the dead and dying lemming could have regional possibilities, for birds and carnivores are susceptible and migrating raptorial birds might conceivably carry infection. The cyclic decline in lemming population is not readily explained and the lemming is an important forage animal, so an infection that is not often easy to recognize and which could fit the case without much stretch of the imagination is worth a little suggestive speculation.

Almost exactly twenty years after publication of the description of the organism we found at Cambridge, a paper published in Sweden by Hulphers in 1911 came to light. In this he described an organism that is undoubtedly a *Listeria*, but I think probably a different species. Struck by the necrosis it produced in the liver he called it *Bacillus hepatis*. Pirie, too, was impressed by the hepatic necrosis caused by his Tiger River organism and called it *Listerella hepatolytica* We, on the other hand, noticed in sections a remarkable infiltration of monocytes in the tissues and in the blood vessels, with massive oedema as well as the necrosis. These lesions might well predicate a toxin, and, like Hulphers and others since, we looked in vain for one. Thus the oedema and the necrosis have not been explained. However, since the astonishing monocyte response has been shown by Stanley to be evoked by a lipid of remarkable specificity, which is not only non-toxic but is not even antigenic, it becomes interesting to speculate whether profound pathological changes may not be produced by inherent substances or secretions of bacteria that are not toxins. But, bearing in mind the special conditions other bacteria require and the initial difficulties they presented, it may yet be that a toxin in the ordinarily accepted sense will be demonstrated for *Listeria*.

Another pathological manifestation in the natural disease is the profuse ascites and frequently pleural and pericardial effusions, which clot firmly in a test-tube. This fluid has not been examined biochemically, as far as I know, but it deserves to be because it contains a massive amount of triple-phosphate crystals. When viewed in a beam of strong light the crystals glint and give an appearance unusual to such exudates; they are so abundant that more than enough of them can be collected for chemical analysis by centrifuging ten to twenty cubic centimetres of exudate. This must indicate some peculiarity in disturbance of host metabolism and of bacterial character worth the knowing.

The mononucleosis characteristic of this disease very naturally attracted most attention and was quickly appreciated, judging by the immediate request for cultures. A monocytic reaction is somewhat characteristic of typhoid fever, tuberculosis, certain streptococcus infections, some protozoon diseases such as amoebic dysentery and trypanosomiasis, and at least one suspected virus disease, but its genesis and function remain unexplained. Beyond saying that the etiology of the syndrome of infectious mononucleosis is still controversial and that an inference of a virus infection is justified in some cases, it is proper to note that *Listeria monocytogenes* has been isolated by different workers from typical cases and, with the support of indirect evidence, it seems possible that more than one cause exists.

Be that as it may, the mononucleosis caused by *Listeria monocytogenes* far exceeds that of any other instance. The circulating monocytes were shown by us to be increased by sublethal infection as much as 6,000 times, and it has been amply confirmed that this selective stimulation never failed to be produced beyond any need of statistical analysis to appreciate it. With it the absolute number of other blood leucocytes was but little disturbed.

The function, nature, and origin of the monocyte are still contentious and the fact that these cells are variously known as macrophages, monocytes, large-mononuclears, histiocytes, clasmatocytes, endothelial leucocytes, and plasmocytes shows an eclecticism indicative of uncertainty. The infection with *Listeria* affords a means of investigation far beyond the otherwise best limits of experimentally induced monocytosis, using tubercle bacilli or olive oil with Sudan III. Many have used *Listeria* with interesting results, but I shall not digress into the histological or cytological evidences produced to support the still varying views. Briefly, the prevailing view is that the monocyte, or certain of these diversified cells, are a transformation of the lymphocyte, though some maintain that there is a reticulo-endothelial hyperplasia or a specific monoblast. In any case, how and why the transformation occurs remain a mystery. There is no present understanding of the process inducing the monocytic response and the observation that ruminants do not produce a monocytosis, though susceptible to infection, has been neglected.

There still remains the all-important question of the function of the monocyte. It is not pre-eminently a phagocytic cell except in a very selective way. It takes up certain dyes peculiarly, it responds specially to lipid fractions of the tubercle bacillus to give histological features to the anatomical tubercle, and it takes up living tubercle bacilli in a moderate way. I have seen monocytes greedily ingest damaged neutrophyl leucocytes in exudates induced by the saliva of *Gastrophilus equi* larvae. Selective phagocytosis is not unusual, for even the familiar "polymorph" will take up carmine in preference to bacteria and one kind of bacterium in preference to another in a mixture. In the original investigations we showed that the monocytes produced by *Listeria* infection would cram themselves with living or dead *Listeria*, both *in vivo* and *in vitro*, when they would refuse almost completely to ingest *Escherichia coli*. Selective phagocytosis is a much neglected subject but it is unlikely that this degree of activity can be the chief function of the monocyte.

The selective stimulation of a monocytic reaction by *Listeria* gained enormously in importance when Stanley discovered in 1949 that a chloroform-soluble lipid extracted from the bacterial cell was alone responsible for it. He also extracted the lipid from the liver of infected rabbits and could not find it in the normal liver. This lipid is not antigenic and is non-toxic, and produced a monocytosis of much the same order as the infection does, but of shorter duration, as might be expected. All this we confirmed extensively. At the time we were trying to produce a Paul-Bunnell agglutination of sheep erythrocytes with *Listeria*. We failed in this, but found that Stanley's lipid behaved as a weak Wassermann antigen. Thus we made an antigenic lipid-protein by precipitating the lipid with human syphilis serum. It is an interesting point, which confirms the specificity of the *Listeria* monocytosis, that some animals immunized with this lipid-protein complex developed a monocytopoenia coincident with production of antibody. The conditions and possibilities of this have not been pursued.

Stanley was quick to appreciate the potentiality of his lipid and showed before we did that animals with a monocytosis produced more antibody than did normal animals. There has been a great interest in Freund's "Adjuvants," using oils and tubercle bacilli to augment the production of immunity, some associating the effect with delayed absorption of antigen and neglecting the monocyte stimulation of these substances and what has been called "allergic irritability." At the same time, there has been a great interest in the origin and site of production of immune antibodies. Much has been done by blockading the reticulo-endothelial cells, by following the uptake of recognizable antigens by monocytic cells and its results, by investigating the concentration of antibodies at the prepared site of inoculation in comparison with that at a distance, by following the fate of lymphocytes with cortisone, and by extracting lymphocytes and macrophages to show their antibody content in response to immunization. Most of this inquiry is not much more than suggestive and there are divergencies in the interpretations.

We have added something to this information and part of the results are ready for publication while other interesting matter is still under investigation. We found it possible to maintain rabbits in a continuous state of high monocytosis by repeated injections of *Listeria* lipid and these rabbits produced four to eight times as much antibody as did normal animals. We used three different types of antigen: whole typhoid bacilli, horse serum, and staphylococcus toxin, all with comparable success. As the lipid is not toxic and not antigenic, there is no interference with the health of the animals and the activity of the cells is not damaged, as they may be by some of the methods used by others. Pleural exudates were induced in immunized monocytosis animals and the washed cells, consisting of 98 per cent monocytes, yielded a higher concentration of antibody on extraction than was in the fluid of the exudate or in the circulating blood. We do not feel justified in concluding yet that antibody is made in the monocyte, because, when antibody from a normal rabbit is injected into a monocytysis rabbit, the collected monocytes contain a greater amount of the passive antibody than is present in the circulating blood. We have also shown that monocytes from a normal non-immune rabbit will take up a considerable quantity of antibody *in vitro*. Thus we do not know yet whether antibody is produced by other cells and the monocytes merely take it up and store it. It is quite evident that this approach opens vast possibilities in the investigation of immunity problems and the function of the monocyte.

There is implicit in these observations an explanation of the primary immediate disappearance from the circulating blood of passively introduced antibody. It raises the additional question whether this antibody stored in monocytes, which may account for the disappearance within twenty-four hours of at least 50 per cent of that introduced, might still be available for use, or might be destroyed in the cell. This is a matter of some importance in the serum treatment of disease and the answer could materially alter the applications of passive immunization. As yet I hardly dare speculate on the possibilities of influencing methods of active immunization, but in dreamy moments I see attractive hazy vistas only to be brought back to the realities limiting opportunity for experimental research.

This is an outline of the story of *Listerella*. Quite evidently a great part of it has been contributed by many workers whose names I have not quoted and a lot of varied detail of interest to specialists has been left out. "There is no man now on earth so artful in mind, nor so mighty in strength, that all these arts should be put upon him alone," and there is some merit in "le premier pas qui coute." It was my habit to keep complete notes of autopsies on all so-called normal animals which died, with bacteriological and histological examination of lesions. The noticing of apparently increased numbers of monocytes in the smaller blood-vessels in these sections is "the Glass Slipper" of this story.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVII : SERIES III : JUNE, 1953
SECTION FIVE

The Postglacial Pine Period*

PIERRE DANSEREAU, F.R.S.C.

POLLEN analysis shows rather a consistent dominance of pines in Eastern North America through a fairly considerable period of time. Potzger (1946) has assembled a number of pollen profiles that cover the Great Lakes area and they are consistent in revealing a well-defined pine dominance (see especially his figures 8, 9, 10, 11, 12, 14, 17, 22, 25). Allowing for the fact that species of *Pinus* are generally over-represented in pollen spectra, whereas some of their usual associates or near-associates (*Acer* and *Fagus* especially) are under-represented, it remains that pines in large numbers did occupy extensive areas for long periods of time.

In his general review of Pleistocene and postglacial biogeography, Deevey (1949) does not hestitate to characterize one of the three major subdivisions of postglacial time as a pine period (see his table 7). He emphasizes that "the resemblance to the European Boreal is too close to be ignored. Evidently in this interval the climate became considerably warmer and drier, and pines, presumably different species (e.g. *Pinus rigida* in New England, *P. banksiana* and *P. resinosa* in the Middle West), found suitable habitats on the poorer soils. It is not implied that pine forests occurred everywhere during this phase. The universal occurrence of large quantities of pine pollen (normally over 50 per cent) must mean that pines occupied at least the poorer soils while deciduous trees were still absent from the richer sites."

Deevey then goes on to contrast the striking regional differentiation during the following period, with the apparent uniformity of the pine period. However, he does not develop the interpretation quoted above and attempts no further description of the vegetation of the Boreal period.

More detailed study, such as that of Cain and his collaborators in southeastern Michigan (Cain, 1948; Cain and Cain, 1948; Cain, Cain, and Thomson, 1951; and Cain, unpublished), indicates the presence of at least three species of pine.

---

*Contribution no. 1003 from the Department of Botany, University of Michigan.

This forces us to face one of the most troublesome contradictions of plant geography: the fact that we must reconcile our extrapolation of present plant requirements into the past with our knowledge of evolutionary processes. It is hard to say which is more surprising, biologically speaking: the great stability (morphologically, at least) of some groups, such as *Gingko* and *Liriodendron* or the great change and multiform differentiation of others, such as *Nothofagus* and *Acer*. It is hard to decide upon, in fact vain to look for, any general rule concerning the balance of conservative and innovating forces within a phylum. There is evidence to show, at least on the infraspecific scale, that both processes are at work. It has been possible to detect them in the Californian closed-cone pines for instance, where there appears to be survival of unchanged old types together with appearance of new, modified ones—both of these phenomena (conservation and innovation) taking place within the changing matrix of the environment. (See Cain, 1944, chap 9, pp. 104–21.)

If we add that physiological change may take place without any outward morphological sign, we can get ample confirmation from well-authenticated present-day instances· morphologically indistinguishable plants exist on different ploid levels and/or in different areas and/or habitats (Clausen, 1951). Such are the ways of evolution, detectable and undetectable, and of course, for the majority of plants, undetected.

May we then legitimately turn to a fossil flora and grade it by assigning indicator value to its components -reconstruct the vegetation itself by assuming that *Fagus grandifolia*, *Pinus*, *Lauraceae* indicate "cool-moist," "cool-dry," "tropical" conditions?

In the first place, it is rather evident, even from contemporary distributions, that the larger taxa offer the least reliable criteria. Genera are usable only if their ecological or bioclimatic limitations within a wide-ranging flora are quite sharp. For instance *Abies* is a better indicator in Eastern than in Western North America. As for species—when they can be positively identified!—their morphological stability may or may not be the outward sign of their physiological (and therefore ecological) stability.

Applying the above criteria to the postglacial pine period in Eastern North America, several explanations remain possible which are outlined below. Two principal hypotheses can be proposed. The first assumes that the three species of pine now widespread in glaciated eastern North America (*P. banksiana*, *resinosa*, *strobus*) had the same ecological requirements in the past as they have today. The second assumes that one or more of them did not have the same requirements.

PRESENT ECOLOGICAL STATUS OF THE NORTHEASTERN PINES

At this time, the jack, red, and white pines appear, ecologically, as a well-graded series consisting of three almost equidistant species in the order mentioned. From *banksiana* through *resinosa* to *strobus* the following requirements increase· moisture in air and soil (mostly the latter), humus content of soil, heat present in atmosphere (climatic, not necessarily edaphic). On the other hand, the light requirement decreases in the same order.

Much of the above has been demonstrated experimentally by Grasovsky (1929), Burns (1933), Shirley (1945), and many others, who confirm the relative positions of the three species along a light or a moisture gradient Although they all require much light (at least in the earlier stages of their life-cycle) and can withstand various intensities of drought, it is also very apparent (a point which is much stressed by the above authors) that competition (especially in early stages of development) is ultimately the most important limiting factor This latter point brings out the fact that in the foregoing paragraphs the term "requirement" has been used rather loosely. Ability to compete is hardly a thing-in-itself. It is the resultant of accumulated capacities, of successful adjustments, and varies a good deal in any and all species as they are exposed to different environmental conditions Actually, the *ecological position* and the *potential ecological strategy* of the three species are only in part determined by their "requirements" in any strict sense of the word (Dansereau, 1952).

My principal concern here is the role played in natural communities by all three species: where they stand in relation to one another and in relation to other species of similar life-form and of neighbouring ecological status It will turn out that each one shows certain requirements, tolerances, and capacities of utilizing the total resources of the environment. The various levels of efficiency attained will permit it to gain and to hold a certain ecological position in a habitat or general climatic environment that may be stable or changing

Summarizing the ecological position of the three pines, it would seem to be generally agreed among ecologists and foresters who have wide field knowledge that they line up as follows.

Pinus banksiana (jack pine) ranges farthest north, into the subarctic taiga (lichen woodland), where it is sometimes a member of the prevailing open climax community, associated as a minor component with spruces; there, as well as in the south, it grows on rocky outcrops, mostly acid (granite, gneiss, quartzite), occasionally dolomitic ("limestone pavement" of Manitoulin Island and Bruce Peninsula), and on sand plains. These sites are relatively dry and have little organic content. When the stand becomes at all dense, the species does not produce viable seedlings any more and one of the other pines, or spruce, or red maple sets in. Most remarkable is adaptation to fire: the jack pine's closed cones can survive a fire and the vast clearings that follow are favourable to its re-establishment notwithstanding the burning of humus at soil surface.

Pinus resinosa (red pine) is altogether the least successful of the three *at this time*. Its northern limits very nearly coincide with those of white pine and they are also very close to those of many ecologically important broadleaf trees (notably sugar maple). In fact the whole range of this species is within Nichols' (1935) "hemlock–white pine–northern hardwood" region and E. Lucy Braun's (1950) "beech–maple–hemlock" region. Red pine occurs on sandy and rocky (acid) substrate but not very often in extensive pure stands like the other two. For the most part it is associated with *P. banksiana* or *Populus tremuloides* on the drier and more open situations, and with *P. strobus*, *Acer rubrum*, or *Quercus rubra* on the more mesic sites. A canopy of about 50 per cent coverage seems fatal to its regeneration.

Pinus strobus (white pine) extends farther south than red pine, especially along the Appalachians at mid-altitude.[1] The moisture range of the sites it is found on is quite remarkable: from dry sand plains to the edges of bogs and arborvitae swamps. It seems to be absent from truly calcareous soils, whereas it does best on deep deposits of sand or not-too-coarse gravel. It is the only one of the three species which can achieve very dense pure populations of mature individuals. Under such conditions, although it is incapable of producing viable seedlings, it can maintain itself for very long periods of time. Although this is more in evidence in some areas than in others, it is generally agreed that white pine is not a climax dominant anywhere in Eastern North America at this time (Oosting, 1948).[2]

It thus appears that the three species are equipped for different functions, climatic and ecological. The white pine has the greatest ecological amplitude and is inserted into many different lines of succession (the bog hydrosere and the dune xerosere). The other two

[1] Outlying colonies have been reported in Mexican and Guatemalan highlands (Miranda and Sharp, 1950; Sharp, 1950).

[2] It is not argued that white pine is not an occasional or minor member of the maple–beech or maple–beech–hemlock climax community. But the view is taken here that extensive stands of pine were and are subclimax or at least edaphic and not climatic climaxes. Potzger (1946) has discussed this question in some detail.

are confined to the dry part of the ecological spectrum; and *P. resinosa* is further restricted to a rather narrow band within that.

HYPOTHESIS I: UNCHANGED REQUIREMENTS

If, in the course of the Pleistocene, the three pines acquired or had already acquired their present physiological adjustment to their environment, and had settled into their present ecological niches, they can be expected to have behaved during postglacial time as they do now. It will therefore be expected that their local abundance and association with other species will convey the same meaning as it does today in terms of both climatic and edaphic trends. If it can be shown that they did perform some function that they are now incapable of fulfilling, it follows that their (unchanged!) requirements found *another* adjustment to environmental conditions essentially different from any to which they are exposed today.

Under this present hypothesis of unchanged requirements four different situations (hereafter called cases A, B, C, and D) would explain the dominance of pine.

Case A: Long-drawn Succession

Whereas during ice advances the cliseral movement proceeded through the contamination and eventual replacement of communities by other communities of equal seral status (climax by climax, serclimax by serclimax, pioneer by pioneer), during ice retreats progression over newly liberated areas, although uneven in rate, largely proceeded through occupancy by many seral stages, at least on the "better" sites.[3] In other words, while the southward movement allowed a community with high requirements to move directly onto a highly differentiated soil (spruce–fir moving into beech–maple), it was necessary for that same community on its way back north, to have other communities before it build up the soil, unless, indeed it could do so itself.

Many questions arise in this connection which can only be hinted at for the moment: How well can biological replacement of beech–maple by spruce–fir keep up with the pace of a "deteriorating" climate? How soon can the replacement of spruce–fir by beech–maple catch up with climatic "improvement"? There would always seem to be a lag: survival of relict deciduous forest during the ice advance; slowness of edaphic build-up during the ice retreat. If this latter were extremely prolonged, true climatic control would not set in

---

[3] E. L. Braun (1950) refers to the well-drained upland as "average" topography.

(at least not in a general way and over most of the area) within the duration of the climatic cycle itself. Braun (1950, 1951) believes that there is evidence even today that many Tertiary species, disturbed by glaciation, have not yet regained their former positions and are at this time checked in their expansion not so much by climate as by the relative immaturity of soils. Obviously this line of reasoning can be applied only to climax or near-climax species.[4]

In Case A, then, *pine dominance* (most likely white pine) *is explainable in terms of a prolonged succession which never attained its* (deciduous?) *climax status*, and *the apparent boreal pine climax is in reality a long-enduring subclimax.*

The persistence of vast areas of white pine to this day and the readiness with which they are supplanted by broadleaved trees when released by lumbering fit this case quite well.

Case B: Dry-Cold Climate

If there is postulated a former climate which was approximately that of the Great Lakes today in temperature fluctuations, whereas the total precipitation is assumed to have been much less, then no area in the East duplicates such conditions today. In fact, as long as much ice still plugged the Great Lakes themselves, it is not unreasonable to suppose more pronounced continentality in the climate. Whereas today there is practically no climatic savana (or woodland) between the grassland and the forest in Eastern North America, it is by no means unusual for such an intermediate formation to occur. Pine savana is almost constantly present in Western North America where desert, grassland, or steppe abuts onto the forest, whether the latter be broadleaved or needle-leaved. It makes a considerable difference in our interpretation if we do not accept "pine" as necessarily indicative of "forest," but recognize its potential role as a savana tree. Of the three being considered here, it seems that *P. banksiana* is indeed exactly that in the Canadian taiga, where, however, it is of minor importance as a true climax species. Throughout its range, whatever its successional status, it is most often a member of an open savana or park-like formation. *P. resinosa* is perhaps even more markedly a savana tree.[5] It is worth pointing to the role played by the closest relatives of these two species. *P. contorta* and its many segregates in Western North America (including *P. murrayana* characteristic of the "Hudsonian" belt in the Sierras) are very much like *P. banksiana* and quite frequently grow in very open stands. Even more striking is *P. ponderosa*, in many ways very similar to *P. resinosa*, which on the western edge of the Great Plains seems to offer a picture of the *P. resinosa* savana or savana woodland that could very well have bordered the Great Lakes in postglacial times.

---

[4] That is to say, to those that are obligate mesophytes and not to those which persist in climax, but become established earlier and thereby show some independence of degree of maturity of the soil.

[5] I have discussed the importance of structure in vegetation and attempted to outline its significance and variations at some length in another paper (Dansereau, 1951).

In Case B, *low precipitation induced a climax savana formation of jack or red pine.* It is entirely possible that increased heat without proportionately increased precipitation resulted in a shift from the dominance of jack to red pine without much change in vegetation structure.

Case C: Greater Illumination

Low light intensity at forest floor level seems to be primarily responsible for failure of white pine to maintain itself, whether under its own shade or that of other species, needle-leaved or broadleaved. Amount of light in the forest is of course a function of amount of radiation that penetrates through the atmosphere. At the present time the mean cloudiness in the Great Lakes is quite high (Brooks, Connor, *et al.*, 1936). Table I permits a comparison with other parts of North America. It will be seen that light availability is highest in the Sierra Nevada where the most truly mesophytic and most shade-tolerant of

TABLE I

MEAN CLOUDINESS IN VARIOUS AREAS, WHERE PINES ARE OF ECOLOGICAL IMPORTANCE

(Brooks, Connor, *et al*, 1936)

Region	Southern Appalachians	St. Lawrence	Great Lakes	Colorado	Sierra Nevada
Pinus spp.	*virginiana* *echinata* *taeda* *rigida* *strobus*	*banksiana* *resinosa* *strobus*		*contorta* *ponderosa* *flexilis*	*contorta* *ponderosa* *flexilis* *lambertiana* *monophylla*
Mean cloudiness January	6–7	6–7	6–8	5–6	4–6
July	5	5–6	4–5	4	1–2

all American pines is found: [illegible] authentic forest climax species. It also [illegible] white pine.

Case C, then, offers the possibility that the intolerance of *Pinus strobus* could have been overcome in the past if greater illumination had prevailed.

Case D: Higher Climatic Anomalies

Today the Great Lakes area, as compared with the Central Plains, shows relatively equable conditions. The North American maximum range of temperatures (46°C. and more in Brooks, Connor, *et al.*, 1936) lies in the Mackenzie district roughly between 55° and 65° Lat. N and 95° and 125° Long. W. This trend is gradually and almost regularly attenuated to the southeast on the axis of 100° Long. W where the range of 24–30° lies between 35° and 42° Lat. N. This same range (24–30°) is then oriented in a northeasterly direction on an axis perpendicular to the Great Lakes–St. Lawrence between 42° and 48° in the western Great Lakes and 50° and 55° in Newfoundland and Labrador. The middle and upper St. Lawrence, however, have 30–32° ranges. This latter is the result of a thrust from the Mackenzie centre[6] which is quite analogous to the southward push along the 100th meridian.

I venture to suggest that in postglacial times these anomaly lines were displaced and that the bulge which surrounds the Great Lakes must not have existed at all for quite some time posterior to deglaciation. It is therefore entirely possible that the high anomalies that dominate the taiga may have existed at more southerly latitudes where they need not have involved mean minima very much lower than those which prevail today. Such large-scale fluctuations, which may well have also been irregular, can be thought of as offering a background to plant succession, such as does not exist today, at least on the same scale, in the Great Lakes region. What might have been involved is a state of flux between the conditions essentially favourable to the hardwoods and those essentially favourable to spruce-fir. Whereas the white pine community is now subclimax to hardwoods, and occasionally (as in the Gaspé Peninsula and northern Ontario) to spruce–fir, it may possibly have held its own in an area that was alternately favourable to the one and to the other and never for very long to either one.

---

[6] Hare and Montgomery (1949) have shown how the winters of the Hudson Bay are truly continental as a result of frozen water and oppose no resistance to the above-mentioned movement.

Case D *supposes* Pinus strobus *to have been climax in areas of long-continued climatic variability or of rapidly alternating periods of different climates.* The intricate patterns of individual climatic factors and the sharp gradients that they reveal in the central lower peninsula of Michigan and the plateaus of Pennsylvania suggest a possible relation of white pine stands (primevally well developed there) to climatic instability.

The Shifting Order of Past and Present Vegetation

If one postulates that the three pines we know today are descended from ancestors that may or may not have been morphologically identical to them but were somewhat physiologically (and therefore ecologically) different, it would amost seem that there are endless possibilities.

This will hardly be so, however, in any one line of descent, as it is unsafe to assume too much or too many changes. Palynological data themselves provide us, after all, with a generally coherent picture of plants in the past that are associated with the same other plants with which they grow today: the beech and maple are commonly found together, so are the birches and alders, so are the oaks and chestnuts, the spruce and fir.[7] An over-all view of fossil pollen aggregates rather points to a certain cohesion of the phytosociological units which can only be due to a compatibility founded on both similar and complementary requirements In the light of this interpretation, Hypothesis I answers the principal questions raised by the position and relative abundance of the pines.

It is, however, permissible to consider the possibility of evolution of the modern pines from ancestors that differed from them in some respect, although this divergence cannot be postulated to involve any one or all possible ecological functions. Our best estimate of the kind, degree, and direction of deviation which modern pines underwent in postglacial times can be made on the basis of their previous history. This approach has already been implicitly suggested above when a comparison was made between *P. strobus* and *P. lambertiana*, and between the other eastern pines and their western vicariants. A further step would consist in reconstructing as accurately as possible the Pliocene environment and in working back in the Tertiary and

---

[7] This in itself is hardly conclusive since we cannot be sure of the time at which dominance was achieved by one or more species. At the time of the rise of the Angiosperms, various species of *Fagus*, *Nothofagus*, *Quercus*, *Acer*, etc , displaced entirely or in part the reigning *Podocarpus*, *Tsuga*, *Pinus*, etc., and there must have been later shifts among the Angiosperms themselves.

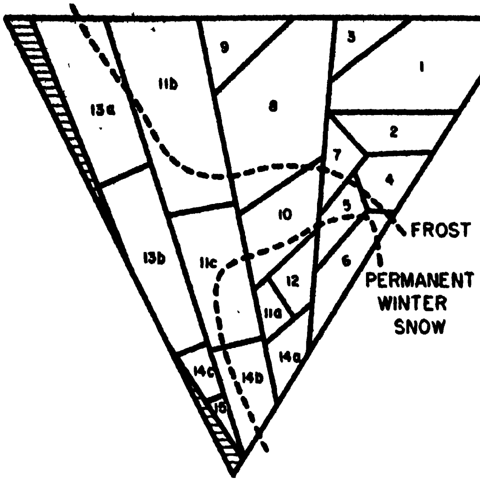


FIGURE 1. A world scheme of correlation of plant formations and major climatic influences (modified from Dansereau, 1951, Fig. 5). Extreme of warmth and moisture is in upper right-hand angle, extreme of warmth and drought, upper left-hand angle; extreme cold and low precipitation, lower angle. The figures in the divisions refer to Schimper and von Faber's (1935) formation types, as follows (examples in parentheses):

1, Tropical rain forest (Amazon). 2, Subtropical rain forest (New South Wales). 3, Monsoon forest (Burma). 4, Temperate rain forest and cloud forest (New Zealand). 5, Summergreen deciduous forest (Eastern North America). 6, Needle-leaf evergreen forest (northern Ontario). 7, Evergreen hardwood forest (southern France). 8, Savana woodland (northeastern California). 9, Thorn forest and scrub (northeastern Brazil). 10, Savana (central Brazil). 11a, Scrub steppe (Wyoming). 11b, Steppe (S. Dakota). 11c, Prairie (Iowa). 12, Heath. 13a, Dry hot desert (Sonora). 13b, Dry warm desert (Mojave). 14a, Cold woodland (Labrador hinterland). 14b, Grassy tundra (Seward Peninsula). 14c, Rocky tundra (N. Baffin). 15, Cold desert (N. Greenland). Horizontally striped areas have no vegetation. The approximate limits of frost and of permanent winter snow are shown.

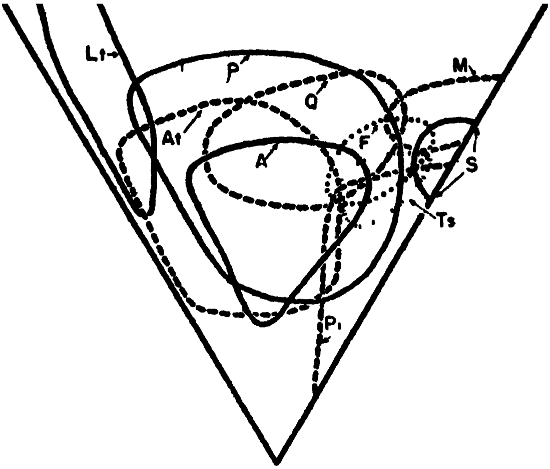


FIGURE 2. Superimposed upon Fig. 1 are the approximate limits of several genera or smaller taxa, with which the present discussion is concerned Their distribution in North America is outlined with respect to the major formations which they span. They are as follows:

A —*Andropogon gerardi*
At—*Artemisia tridentata*
F —*Fagus*
Lt—*Larrea tridentata*
M—*Magnolia*

P —*Pinus*
Pi—*Picea*
Q —*Quercus*
S —*Sequoia*
Ts—*Tsuga*

Mesozoic to the initial contest between the needle-leaved evergreen conifers and the broadleaved deciduous Dicotyledons.

We can view some of the highlights of the evolutionary sequences, the morphological transformations, the geographic isolations that took place in that long period. It is possible to follow the development of certain phyla—and even genera—quite well, but difficult to reconstruct the entire plant communities, the whole vegetation matrix that served as a very real background (with all its accumulated and cumulative pressures), to evolutionary processes.

No documented synthesis of the vegetation of North America in pre-Pleistocene times is available, although many regional studies have been made. However, the floristic character and climatic limitations of some of the important vegetation units are known and a scheme showing the order of replacement of the one by the other can be devised. Also some estimate is possible of the shifts in requirements that enabled certain species to become dominants and thereafter to maintain or lose that position.

This relationship is pointed up in Figs. 1 and 2. Fig. 1 (somewhat modified from Dansereau, 1951) shows a world scheme of the principal climatic vegetation types and Fig. 2 shows the range of certain plant groups with which the present discussion is concerned through two or more formations.

The principal vegetation units involved in the North American late Tertiary, Pleistocene, and Recent are the following: (1) *temperate rain forest* (and its montane equivalent, cloud forest); (2) *redwood forest* (also a modified temperate rain forest); (3) *hemlock–cedar forest* (a further attenuation of the rain forest complex); (4) *deciduous* or *summergreen forest*; (5) *needle-leaved forest*; (6) *oak-openings* (a savana woodland); (7) *pine woodlands*; (8) *sagebrush*; (9) *creosote bush*; (10) *grassland.*

These ten types will not be described here. They are well outlined in Shantz and Zon (1924), while the major vegetation-types to which they belong are described and justified in Schimper and von Faber (1935). Our interest lies in their points of contact and their potential for moving along a climatic gradient in a certain order. Table II shows four sequences, each involving three stages of replacement under changing climates.

It will be seen in both Fig. 1 and Table II that *Pinus* not only occupies a central position but also ranges more widely than any of the others. Considering, then, the present position of pines, their occurrence at various levels in pollen diagrams and their occasional dominance; considering also the origin of the regional formations within which they grow or by which they are eventually displaced, what can be construed concerning their past requirements if the latter are assumed to have been any different from their present ones?

TABLE II

Some of the Principal Cliseral Alignments in North America at the Present Time

Magnolia. (warm-moist)	*Fagus* (cooler)	*Tsuga* (cooler)	*Pinus* (cool-dry)
Sequoia . (warm-moist)	*Quercus* (drier)	*Pinus* (cooler)	*Artemisia* (cold-dry)
Larrea (hot-dry)	*Artemisia* (cooler)	*Pinus* . (moister)	*Picea* (cold-moist)
Artemisia (cold-dry)	*Andropogon* (moister)	*Pinus* (moister-colder)	*Picea* (cold-moist)

Hypothesis II: Changed Requirements

Three cases are outlined below, if ***Pinus banksiana*** and/or ***P. resinosa*** and/or ***P. strobus*** emerged from the Pliocene with sensibly different genetic make-ups than those which they now possess. Whereas, under Hypothesis I, genetic identity was assumed and exposure to presently non-existing environment was postulated, under Hypothesis II genetic difference is assumed and an explanation will be sought primarily in terms of exposure to presently existing and familiar environmental conditions. It will be obvious that ***differences in both heredity and environment*** will necessarily have existed at some time in the development of each one of the taxa concerned. For greater convenience the ancestors of the three northeastern pines will be called *P.* "prebanksiana," *P.* "preresinosa," and *P.* "prestrobus," as the assumed differences potentially have specific importance whether or not they were accompanied by morphologically visible features.

Case E: Greater Shade Tolerance

Pinus "prestrobus," having diverged less from ***P. lambertiana*** or other more equable-climate species from which it was derived,[8] was able to reproduce in the shade. If the analogy with ***P. lambertiana*** has any value, however, and if the latter has retained more of the ancestral qualities of the stroboid line of descent, because it has been exposed more constantly to equable conditions, it will be noted, on the other hand, that it does not achieve greater abundance. This, however, is in part a function of the degree to which its responses (requirements and tolerances) overlap those of its associates (*Pinus ponderosa*, *Pseudotsuga taxifolia*, *Abies concolor*, *Acer macrophyllum*, and, locally, *Sequoia gigantea*). Essentially *P.* "prestrobus" was facing a different situation, its competitors, with the exception of hemlock, being broadleaved-deciduous. Shade tolerance here may not have been enough as these others have an entirely different (and more advantageous?) seasonal rhythm; their root system, the physico-chemical nature of the litter they produce, and the soil profile which they eventually initiate could all combine to outweigh an equality of response to the light factor. It would seem fairly clear in this respect that *Tsuga canadensis* (which is admittedly also very shade-tolerant, in fact quite as much as or more so than the hardwoods) is less at a disadvantage because of these other factors than *Pinus strobus.*

---

[8] Or indeed to which it gave rise!

Case F: Greater Drought Tolerance

Pinus "preresinosa" in order to maintain its dominance would have had, like *P. prestrobus*, to be more tolerant of shade. Barring this, however, it could have been dominant (as suggested in Case B) if the climate had been favourable not to closed forest, but to savana or woodland. This is the situation under which *P. ponderosa*, its relative,[9] operates most successfully. Therefore, if it had been somewhat more drought-resistant, *P.* "preresinosa" could have held its own.

P. resinosa is now, of course, well adapted to edaphic drought, in fact it occurs on flat Potsdam sandstone slabs and on overdrained sandy hills. It is however almost wholly confined to areas of more than 30 inches of annual rainfall and less than 20°C. mean July temperature. Its western counterpart shows no such narrowness: its lower limit is very nearly 20 inches of annual rainfall whereas its minimum temperature requirements are close to 23°C. July mean temperature (Stage, 1952).

Case G: Greater Moisture Tolerance

Compared to their vicariants, *P. contorta* in western North America and *P. sylvestris* in Europe, the jack pines of eastern North America are singularly depauperate. Two species are known: *P. virginiana* now almost entirely south of the glacial border and *P. banksiana* entirely north of it. They do not come in contact anywhere. Neither is variable to any extent, certainly in no measure comparable to the other two mentioned above. Moreover, the amplitudes of both of the former are tremendous. Climatically they range from the mild maritime conditions of the Pacific Coast and the Baltic to the subalpine heights of the Sierras and the Alps. Ecologically they occur in the bogs of Oregon and California at various altitudes to the bare granite domes of the Sierra Nevada and from the moors of Scotland to the dry valley floors of the upper Rhone in Switzerland. Quite apart from being present at both ends of the ecological spectrum (hydrosere and xerosere), in many parts of Europe and Western North America these pines actually form forests. It is true that these are not often of great density but they are nevertheless forests and not woods, parks, or savanas.

---

[9] The relationships of the pines are now being tested by breeding (Heimburger) and by chemical determination of their resins (Mirov) and some unexpected affinities are being revealed.

It is easy to see how this same pattern may once have prevailed in *Pinus* "prebanksiana" and how it might, in such a case, have achieved and maintained dominance.

CONCLUSIONS

The foregoing pages raise more questions than can be answered at this time. They also accumulate so many suppositions that speculation far outweighs the concrete evidence, and implications are more numerous than conclusions. In an attempt to evaluate a palynological problem, it has been necessary to question all existing interpretations and also to restate some general principles of both ecology and plant geography which do not have a satisfactory grounding at this time.

The basic contradiction which taints much floristic and bioclimatic investigation is here brought into the open. The seven cases discussed under the two hypotheses are probably not the only alternatives that would satisfactorily explain the pine period.[10] I hope that others will be proposed. Nor do I think that they are mutually exclusive. It is more likely that, when we have learned more concerning some of the details and arguments mentioned here, some combination of these cases will provide the most satisfactory explanation.

---

[10] No mention was made of *Pinus rigida*, *P. echinata*, and *P. virginiana*, all of which slightly overlap in area with *P. strobus* and whose cliseral movements may have involved them in the "pine period." Their climatic (not edaphic) moisture requirements seem to be very high. But they too, of course, could have had different requirements in the past.

REFERENCES

BRAUN, E. LUCY (1950). Deciduous forests of Eastern North America. Philadelphia, Toronto; Blakiston.

BRAUN, E. LUCY (1951). Plant distribution in relation to the glacial boundary. Ohio J. Sci., **51**: 139–46.

BROOKS, C. F., CONNOR, A. J. *et al.* (1936). Climatic maps of North America. Cambridge: Harvard University Press.

BURNS, G. P. (1933). A study of natural reproduction in Vermont forests. II. The effect of thinning on white pine reproduction. Vt. Agr. Exp. Stat. Bull., **354**: 1–24.

CAIN, STANLEY A. (1944). Foundations of plant geography. New York: Harper.

——— Pollen profile of a lake in the Edwin S. George Reserve, Mich. Unpublished.

——— (1948). Palynological studies at Sodon Lake. I. Size-frequency study of fossil spruce pollen. Science, **108** (2796): 115–7.

CAIN, STANLEY A. and CAIN, LOUISE G. (1948). Palynological studies at Sodon Lake. II. Size-frequency studies of pine pollen, fossil and modern. Amer. J. Bot., **35** (9): 583–91.

CAIN, STANLEY A., CAIN, LOUISE G., and THOMSON, G. (1951). Fossil pine pollen size-frequencies in Heart Lake sediments, Oakland County Michigan. Amer. J. Bot., **38**: 724–31.

CLAUSEN, JENS (1951). Stages in the evolution of plant species. Cornell University Press.

DANSEREAU, PIERRE (1951). Description and recording of vegetation upon a structural basis. Ecology, **32** (2): 172–229.

——— (1952). The varieties of evolutionary opportunity. Rev. Can. Biol., **11** (2): 305–88.

DEEVEY, E. S., JR. (1949). Biogeography of the Pleistocene. Bull. Geol. Soc. Amer., **60**: 1315–416.

GRASOVSKY, A. (1929). Some aspects of light in the forest. Yale Univ. Sch. For. Bull., **23**: 1–53.

HARE, F. K. and MONTGOMERY, M. R. (1949). Ice, open water and winter climate in the Eastern Arctic of North America. Part I (F. K. HARE). Distribution of winter temperature over the Eastern Arctic and Sub-Arctic. Arctic, **2**: 79–89.

MIRANDA, F. and SHARP, A. J. (1950). Characteristics of the vegetation in certain temperate regions of eastern Mexico. Ecology, **31** (3): 313–33.

NICHOLS, G. E. (1935). The hemlock–white pine–northern hardwood region of Eastern North America. Ecology, **16**: 403–22.

OOSTING, HENRY J. (1948). The study of plant communities. San Francisco: Freeman.

POTZGER, J. E. (1946). Phytosociology of the primeval forest in central-northern Wisconsin and upper Michigan, and a brief post-glacial history of the Lake Forest formation. Ecol. Monogr., **16**: 211–50.

SCHIMPER, A. F. W. and VON FABER, F. C (1935). Pflanzengeographie auf physiologischer Grundlage. Two vols. Jena: Fischer.

SHANTZ, H. L. and ZON, R. (1924). Natural vegetation. *In* Atlas of American agriculture. U.S. Dept. Agr.

SHARP, A. J. (1950). The relation of the Eocene Wilcox flora to some modern floras. Evolution, **5** (1): 1–5.

SHIRLEY, H. L. (1945). Reproduction of upland conifers in the Lake States as affected by root competition and light. Amer. Midl. Nat., **33**: 537–612.

STAGE, ALBERT R. (1952). Growth determinants of ponderosa pine. Unpublished term paper, Department of Botany, University of Michigan.

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVII : SERIES III : JUNE, 1953
SECTION FIVE

Life's Progressive Orderliness based on the Binary Statistic of Genic Alleles

A. H. HUTCHINSON, F.R.S.C.

THE eminent physicist Erwin Schrodinger in his series of lectures on *What is Life?* presented at Dublin and published by the Cambridge University Press (1951) states: "The unfolding of events in the life cycle of an organism exhibits an admirable regularity and orderliness, unrivalled by anything we meet in inanimate matter. We find it controlled by a supremely well ordered group of atoms (the gene). Moreover we conclude that the dislocation of just a few atoms within a group of governing atoms of the germ cell suffices to bring about a well defined change (or mutation) in the large scale hereditary characteristics of the organism." "In the light of our present knowledge the mechanism of heredity is closely related to, nay founded upon, the very basis of the quantum theory." "It now seems to be possible to point out in a more direct manner the connection between quantum jumps and mutations." "These facts are easily the most interesting that science has revealed in our day... an organism's astonishing gift of concentrating a stream of order on itself and thus escaping the decay into atomic chaos—of drinking orderliness from a suitable environment."

The binary basis of biotic order and continuity. Life's continuity is not that of a smoothly flowing or otherwise turbulent stream; it is the quantisized continuity of the shuttle in the loom or in the badminton game which alternates between two poles; the reversible reactions are dependent upon the reciprocal action of opposite forces or co-actors. Again, life's continuity is frequently attained by the cyclic principle of the wheel or spiral where the "quantum jumps" of "to and fro" are smoothed but none the less real. To illustrate: the interaction of male and female reciprocals; the sperm and egg; the homologous chromosomes; the allelic genes; the cyclic alternation of x and $2x$ generations including the reciprocal processes of syngamy and meiosis resulting in the segregation and recombination of genic units. Recent investigations give examples of life's binary phases and alternations in the lowly unicellular algae, yeasts, and bacteria. These are life principles.

The segregation of genes at meiosis is not random; one gene from each allelic pair goes to either of two poles. If chance were in charge there would be an infinite number of poles and any number of like or unlike genes could go in any direction. The number of possibilities of combinations of genes, one from each pair at one of two poles, to form new gametes is quantisized and the distribution is orderly. This orderliness results in definite integral allotments of genotypes. If the hybrids of the first filial generation are heterozygous for n genes, 2^n kinds of gametes are produced, 3^n kinds of genotypes are formed, and 4^n is the least number which makes possible all recombinations. The integral proportions of the genotypes are not fractional; they are specifically 1, 2^1, 2^2, 2^3, 2^4, 2^n where 1, 2, 3, 4, n represent the number of heterozygous alleles involved. The system is one of quantisized orderliness.

Order from order. The orderliness of life processes and products stems from the orderliness of the gene, which is composed essentially of nucleic acid. The orderly configuration of four groups of PO_4, four cyclic five-carbon sugars (ribose); two single-ring nitrogen-containing pyrimidines, and two double-ring purines form the base of the nucleic acid polymer. It is notable that three of these units, adenine, ribose, and phosphate, enter the molecule of A.T.P. adenosine triphosphate, the high-energy compound which facilitates the synthesis of food (Stumpf, 1953). The definite locus of each allelic gene has further significance, known as "the position effect," evident in the phenotypic expression and relative distribution of genes. Again to quote Schrödinger (1951): "Whether we find it astonishing or whether we find it quite plausible that a small but highly organized group of atoms be capable of acting in this manner, the situation is quite unprecedented, it is unknown anywhere except in living matter." Genes are specific in their direction of the final composition of the important protein molecule.

Comparison of the genic orderliness with "chance" occurrences. The odds against holding any specified hand of cards, for instance 13 spades, are 635, 013, 559, 559 to 1. On the contrary the repetition of the Hapsburg lip has continued in regular sequence for centuries and Marquis wheat has repeated itself without failure trillions of times. If the same "chance laws" of distribution within the nucleoprotein of the gene were to hold sway, the several thousand atoms would have a variability such that repetition could not be expected within the time period of the planets. There would be no species of organisms—nothing but a cosmos of freaks.

Mutation, a quantisized genic change. Recent investigation, particularly on the nutrition of Neurospora (Beadle, 1945), demonstrates that gene mutations are not random and in all directions. On the contrary they are in series, step-wise, and limited in degree; there is a gene for each enzyme and consequently for each step in the metabolic process. In addition, the well-known feature holds that mutation is reversible and the rate in either direction is specific, under constant conditions. Change of conditions, such as treatment with X-ray or with colchicine, gives a general increase or decrease in rate but does not affect the relative rates. Survival values may be changed, however. In each case there is regularity, orderliness accompanied by flexibility and selection.

Automatic control and feed back. Recently automatic control and "feed back" have become "conspicuous and widely felt in the mechanics of today"; in thermostats and electric clocks for instance. To quote from Ernest Nagel (1952): "Every operating system from a pump to a primate exhibits a characteristic pattern of behavior and requires a supply of energy and a favorable environment for its continued operation." Further reference to the genic control of the energy releasing A.T.P. may suffice to emphasize the universality of basic "automatic" control in organisms. Consider the energy expenditure of a humming bird in flight and as it hangs pendent at night—a self-adjusting, "automatically" regulating control which is specific to humming birds and varies in rate from one to twenty times the basal rate.

Orderliness versus chance. "The solar system shows so many regularities both in its dynamical and its physical properties that it cannot have been formed by chance. La Place estimated that the odds against these regularities being due to chance was more than 2×10^{-14} to 1" (Jones, 1951). "In the formation of crystals the chances of a nucleus of critical size being formed by random motions of molecules on the surface . . . if expressed as a decimal turns out to have a thousand noughts between the decimal point and the first digit different from nought for a supersaturation of one per cent" (Burton, 1951). What then is the probability that random forces may account for the formation of desoxyribonucleic-acid crystals and for their specific activities? It is estimated that the age of the universe does not give sufficient time for the origin, growth, and repeated reproduction of a single mammal species by chance.

The statistic of the gene. Schrödinger speaks of "our beautiful statistical theory of which we were so justly proud because it allowed us to look behind the curtain to watch the magnificent order of exact physical law coming forth from molecular disorder." Biologists and others, *following the mathematician*, have utilized the infinity extension of the binomial law in attempts to explain the "normal-distribution" of population classes: attempts have been made to study the genetics of human populations without reference to ancestry; the best that can be expected for any individual is an approach to the law of random distribution within the limits of standard deviation; the best that can be attained is to shift the population mean by mass selection, without permanence. Real progress has resulted from recognition of the genes as quantisized units giving origin to particular genotypes. DeVries, while attempting to improve the sugar content of sugar beets, discarded the Galtonian approach in favour of the quantisized Mendel's Law. Nilson-Ehle, when confronted by multiple factors in his investigation of quantitative characters in wheat, used the specific binomial coefficients 1: 2: 1; 1: 4: 6: 4: 1; and 1: 6: 15: 20: 6: 1 to standardize the distribution of his quantisized wheat classes for colour and for size. The most successful analysis of additive multiple-factor inheritance has utilized the step-wise series of binomials indicated by Pascal's triangle in analysing genetic classes. Successive steps give values for additional mutational factors. A paper is in preparation which exemplifies this principle of definite proportions in genetics.

Another paper with graphs is in progress to demonstrate the orderliness of genetic equilibria which are attained toward progressive establishment. Two features may be noted: (1) Successive mutations produce step-wise changes in the proportions of genotypes within a population; these changes are quantisized numerically in time and in direction. (2) There is a remarkable orderliness in the progression of mutant containing genotypes as indicated by the symmetry of the curves charted.

CONCLUSION

The orderliness of genes; the cyclic position of the atoms within the quantisized subgroups of their polymerized molecules; the reciprocal reversibility of their bilaterally interchangeable phases as oxidation reduction, hydrogel hydrosol, euchromatin heterochromatin; their capacity for autocatalysis and reduplication conditioned by "selectivity" of environmental constituents; the principle of "feed back" giving control to the dynamic equilibria of life phenomena; their ability to "feed forward" by projecting reduplicated and recombined groups of genes to succeeding generations; their mutative flexibility—

these features give life "a most regular and *lawful activity, with* a durability and permanence that borders on the miraculous" (Schrödinger).

Man holds millions of cards Does he know how to play them?

REFERENCES

Barton, W K (1951) The theory of crystal growth Penguin Books

Beadle, G W (1945) Genetics and metabolism in Neurospora Physiol Rev **25** 643–63

Jones, H S (1951) The origin of the solar system Endeavour **10**, 39 119–25

Nagel, Ernest (1952) Automatic control Scientific American, Sept : 44–7

Schrodinger, Erwin (1951) What is life? Cambridge University Press

Stumpf, Paul K (1953) A T P, Adenosine triphosphate Scientific American, April 85–93

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVII : SERIES III : JUNE, 1953
SECTION FIVE

The Implications of Recent Advances in Medical Bacteriology

E. G. D. MURRAY, F.R.S.C.

THE phrase "advances in medical bacteriology" might imply a catalogue of the newer methods claimed to be successful in the diagnosis, treatment, and prevention of infectious diseases. This might have an appeal to a society of practitioners in the various branches of clinical medicine, especially were it in the form of a categorical interpretation of the results in highly technical laboratory investigations But this simple though laborious utilitarian treatment of the subject, however complete it might be made, would of necessity assume the character of an obsequious commentary.

So it seems to me more suitable to the occasion, and of better purpose, to search for trends indicated by newer knowledge, with recognition of gaps and inherent difficulties, which will allow of inferences and bring forward problems. Furthermore, the failures in applications may thereby cease to be disappointments and become instead a clearer indication of needed investigations. It is important to take this approach, partly not to be wrong but just as much to try to avoid being "right for the wrong reasons."

Everyone will agree that the outstanding feature of medical history in the past decade is the use of sulphonamides and antibiotics. These are products of the bacteriology laboratory and have profoundly changed the significance of many infectious diseases. They have permeated almost every branch of medicine, revising concepts and techniques, and have caught the imagination of the public at large. The extensive and sometimes wild uses they have been put to have revealed their value and their limitations and have introduced unexpected problems.

Sulphonamides have been eclipsed by Antibiotics without being relegated to the "limbo of lost reputations." The term "wonder drugs" was coined for them in the period of their ascendancy, and of itself expresses the hopes they inspired—hopes which culminated in the unfulfilled vanity of some clinicians, who imagined that soon bacteriologists would no longer be necessary. Many chemically defined variants were synthesized to extend their range of activity and lessen their toxicity. The limits of their effectiveness and the precautions with which they must be used became established and their present application in selected infectious processes was determined. This unsurpassed though restricted effectiveness, the simplicity of their administration, their safety with proper precautions, and their stability, make them the treatment of choice in any but the most fulminant conditions caused by certain micro-organisms. Something is known of their competition for enzymes, making it possible to explain their activity and the development of resistant strains of bacteria, as well as allowing the bacteriologist to inhibit them by the use of para-amino-benzoic acid in specimens to be cultured. However, some observers have noticed their ineffectiveness against very susceptible organisms in cases complicated by the presence of resistant species. To be specific: *Staphylococcus pyogenes* is resistant to most sulphonamides and its presence appears to deprive the patient of benefit of the drug against simultaneous infection with very susceptible *Streptococcus pyogenes*. This has been easily demonstrated in the test-tube by the complete protection of the susceptible *Streptococcus*, from what should be overwhelmingly sufficient sulphonamide, by the presence of resistant *Staphylococcus*. The demonstration opens a field of investigation which might well be illuminating.

At the present time, the treatment of bacterial infections is dominated by the use of antibiotics; so over-reaching is this influence that most patients are treated with one or other of the many antibiotics before ever a diagnosis is made and they are not uncommonly cured. There are claims made of benefit in unbelievable numbers of different diseases and it is very difficult to devise a way of evaluating the situation. No one person can read all the profuse literature to make a critical survey, and I don't know how its volume can be even estimated. An impression of it may be gained by the fact that a drug-house interested in one particular antibiotic has issued a monthly bibliographical list on it for about three years, and in no list are the references numbered in less than hundreds; the largest number I have noticed is 1,348. Even though these lists are not lists of the monthly production of papers, it is impressive that they are quoted to illustrate advertisements in the form of appreciations of the use and activity of a single antibiotic. Fortunes have been expended and re-doubled in the production of these drugs and a perfervid, expensive search is prosecuted to find new ones. Waste-paper baskets are filled with the spate of advertising, samples clutter every physician's mail, sales talk by detail-men is enthusiastic and the competition is frantic. The Nobel Prize has been given in two different years for discoveries of antibiotics. There is no doubt whatever that the interest and value of antibiotics are stupendous, but who can yet know the truth concerning them?

The attitude of a number of medical practitioners to antibiotics resembles that of Galen towards his Samian Clay. He wrote: "All who drink this remedy recover in a short time, except those whom it doesn't help, who all die and have no relief from any other medicine. Therefore, it is obvious that it fails only in incurable cases." The "wonder drugs" give place to the "Miracle drugs." The chemistry of a number of them is becoming known, and, so far, one is commercially synthesized. Their mode of action is largely unknown, so they are characterized by their source, by the range of pathogenic micro-organisms they kill or inhibit (including bacteria, viruses, rickettsia, and fungi), which is spoken of as their "spectrum," and by their toxicity to man and other animals when administered by various routes. For practical purposes the micro-organisms susceptible to each antibiotic are listed and the related therapeutic dose by the tolerated route is given for those of suitable character. But the most noteworthy tendency in recent literature is the emphasis on untoward effects exhibited by all of them, though penicillin might reasonably be excepted. The disadvantage in some is intrinsic toxicity while others interfere by eliminating the normal regional bacteria, which may be classed as friendly, and some encourage local or generalized fungus infections, *Candida albicans* for the most part. There are indications that certain of these effects are linked together and in certain instances may involve vitamins The warning note is a healthy sign of the approaching assignment of antibiotics to their proper place in the treatment of disease, with a reasoned caution replacing an exuberant enthusiasm

What particular diseases any antibiotic will or will not cure is a matter of trial, and this information is purely adventitious. But what physiological processes make pathogenic spirochaetes other than *Leptospira* susceptible to penicillin and what these have in common with a number of unrelated kinds of other susceptible bacteria are questions yet unanswered, because the answers involve knowledge of vital processes not yet appreciated. That susceptibility or resistance to penicillin depends in part on the possession or not of penicillinase is insufficient explanation, because comparable contrasts and similarities are found for any other antibiotic, for which no destructive enzyme has been found. There are, too, overlappings and divergencies in the ranges of action of different antibiotics, as well as synergisms and antagonisms between them, and it is not simply a matter of what one drug won't do another will, but rather of an interference in vital processes, perhaps at different stages or perhaps of different functions. There are intriguing possibilities of identified bacterial breakdown products of some antibiotics (chloromycetin) substituting for amino-acids in the synthesis of proteins and of antibiotics competing with natural substrates in bacterial enzymatic systems. The unexplained stimulation of bacterial and even animal growth by sub-bacteriostatic doses of antibiotics (e g , terramycin and aureomycin) and discrepancies between *in vitro* and *in vivo* effects on pathogenic bacteria, indicate the need for consideration of the drug-to-host as well as the drug-to-parasite relationship. This aspect of the question seems to me the most important as well as the most interesting raised by the study of antibiotics, and solution of it will probably prove most stimulating to new ideas.

Bacteria are living cells possessing all the latent capacity of evolution, which did not cease immediately Darwin described it. The widespread use of different antibiotics is bringing about profound changes in the environment of parasitic bacteria, whether they are pathogenic or not. This is indicated, for example, by the finding of known bacteria in unusual places and by the disappearance of expected kinds from accustomed places. What disadvantages may accrue from the disturbance of synergistic and symbiotic associations are merely hinted at present; among others, deficiencies of Vitamin B and Vitamin K, or the preponderance of *Proteus* in the gut with the suppression of enterococci, are associated with use of an antibiotic. But the influence attracting most attention for the moment is the increase of resistant strains of pathogenic species previously susceptible to a particular antibiotic and there is even development of dependent strains. Whether this influence is of genetic significance or is the redevelopment of an atavistic potentiality intrigues some of us, while others are merely bewildered by the practical implications.

These various considerations stir the imagination. The stupendous life-saving achievement of antibiotics and the new aspect, compared with their historical character, imposed on several ravaging diseases, such as lobar pneumonia, all forms of meningitis, syphilis and others, is enormously encouraging. But there is a sobering effect in the momentous possibility that the situation might conceivably be reversed again. Parasitic and pathogenic micro-organisms evolved in the course of time, with natural selection, achieving remarkable host specificities and great pathogenicity, but occasionally some degree of mutual tolerance or even mutual aid has developed between host and parasite. Even in recorded history, syphilis and tuberculosis have, like other diseases, been seen to undergo astonishing modification in severity and it must be supposed that organisms normal to our bodies now may not always have been so. The struggle for existence imposed by the environmental changes we are inducing rather carelessly and the evident capacity of bacteria to take advantage of their quick succession of generations, may well result in new races of unsuspected characters, and may revive the severity of old known diseases or bring about new ones.

You would call it an unwarranted flight of the imagination were I to predict a return of syphilis to its fifteenth-century fulminance--unlikely because *Treponema* has not been shown to develop antibiotic resistance. Yet there is more than enough to be disturbingly indicative in the prevalence and severity of staphylococcus infections during the past four or five years. Whether there are new diseases developing under our eyes is a question which could be argued without decision. There is much interest in epidemic diarrhoeas caused without doubt by an *Escherichia coli*, indistinguishable from everyone's everyday "B. coli" except by its immunological antigenic configuration. I well remember when nearly all Europeans visiting Egypt suffered from "Gyppy Tummy," although some of the most competent bacteriologists of the time could only find ordinary "Bacillus coli" and no known pathogen. They searched well and were as good as any of their kind today, but antigenic analysis was not developed then to its present state. Is the present troublesome diarrhoea a new disease or an old one better differentiated? The same difficulty is met with in other instances.

There is clearly a growing interest in the structure of the bacterial cell and with it a concern with intra-cellular physiology. For the present I may omit consideration of this subject as beyond the confines of medical interests, but the study of the effects and processes of bacteriophage virus infections of bacteria will one day intrude into the medical field, on the basis of established principles. Not unrelated is the question of bacterial genetics and, though there may still be some uncertainty about actual crossing between bacterial cells in the form of conjugation, there is exciting evidence of what may be loosely called genetic exchange or transference. It has been shown beyond question that single, strictly identifiable, somatic antigenic characters have been transferred hereditarily from one species to another closely related species, in which they did not exist before. The other natural antigenic characters of the recipient species remain undisturbed and, so far, such identifiable marks of distinction can only be transferred one at a time, under closely limited conditions.

It has been claimed elsewhere that crossing explains changed physiological characters and that heritable mutations can be induced by various means. All this is matter of great importance as it involves the previously maintained fixity of immunological types within the species, upon which rests complete identification of bacteria and many epidemiological investigations and concepts. It foreshadows possibilities I hesitate to implicate, but which urgently crave examination in order that we may know whether the process is limited to Types within a species, or may concern nearly related species, or embraces wider taxonomic groups. It is devoutly to be hoped that some crosses, if such they be, turn out mules, lest there prove to be too much too new and too suddenly

Meanwhile we know enough to identify most bacteria, and many rickettsia and viruses, and to recognize the diseases they cause General interest in the now well-known presence of sylvatic plague in the prairie rodents has been lulled to oblivion and only wakens briefly with the sudden appearance of a rat in Alberta Of more recent concern is the invasion of rabies in the wild life and dogs of the North-west Territories, whence it has spread into the neighbouring provinces This preoccupation is perhaps because the cry "mad dog" strikes the same frightening note as "Leper" did in the Middle Ages, but with more justification. Along this line of inquiry, there is perhaps more importance and even urgency in the discovery of tularaemia in the beaver and muskrats, with recognition of its spread through the western provinces and into Ontario, as well as through the border states The spread of this infection in these animals has been recognized only fairly recently, and together with the discovery that *Pasteurella tularense* can persistently contaminate water, it is sufficient to be alarming. The great variety of susceptible hosts, the different arthropod vectors, and the assorted sources of human infections add weight to the warning. The proven arthropod blood-sucking vectors of this disease have a Canada-wide distribution and what others of our large and small wild mammals and birds may be already infected has yet to be discovered.

Closer co-ordination of human and veterinary medicine is evidently most desirable. In addition there is need for extension of the much-neglected study of diseases of our wild life, which should not be confined to fur-bearing animals and game.

I hesitate to embark on the relation of ACTH and cortisone to infection and immunity. There is strong evidence of suppression of antibody production as well as instances of the intensified generalization of infection by their administration. Some maintain that fulminant purpuric meningococcus septicaemia is an expression of damage to the suprarenal cortex but I do not find the evidence so far adduced convincing. Experimental animals are certainly rendered more susceptible to meningococcus infection by cortisone. In the human cases no upset of the potassium and sodium balance has been found yet, and many cases show no damage to the suprarenals at autopsy, though some do; also, the blood pressure is often raised to start with though collapse supervenes later. It might just as well be an initial hyper-active suprarenal cortex with subsequent suppression that is responsible for these fulminant cases and for the fact that some are benefited by cortisone late. I decline at present to become involved in the theory of "Stress" and I almost dare not use the word in any of its several dictionary senses, since in some quarters it is becoming almost synonymous with *disease*, even including infections.

The propagation of viruses in tissue culture represents a realization of the present and a promise of a future which may become equivalent to the pure culture studies of bacteria. The possibilities of rapid isolation and serological typing of at least certain viruses lend hope for more exact epidemiological studies and diagnostic accuracy, which may well lead to great progress in the management of the diseases they produce. The long delay and the uncertainty of recognizing specific immune bodies, developed late in the history of a case, have greatly hampered progress; while diagnosis by infection of susceptible experimental animals has been beyond the means of any but special research undertakings. This is one of several interesting applications of tissue cultures and, although most of them do not yet come within the scope of this review, the possibilities extend beyond the field of animal viruses.

In submitting this review I have not attempted an exhaustive analysis of advances in bacteriology of medical significance and I have purposely left out all questions of immunology. I am fully aware of many shortcomings which those with medical knowledge will readily recognize. I have used my experiences and general information without documenting every statement with citations from the literature, in order to avoid a stilted presentation.

The purpose of the instances selected and the interpretations put upon them is to show that there are principles of wide biological significance to be gleaned from microbiological studies. Much has yet to be learned before we can "expound the forms of savage creatures and their deadly injuries which smite one unforseen and the countering remedy for the harm."

TRANSACTIONS OF THE ROYAL SOCIETY OF CANADA
VOLUME XLVII : SERIES III : JUNE, 1953
SECTION FIVE

The Use of Plant Material in the Recognition of Northern Organic Terrain Characteristics

NORMAN W. RADFORTH

Presented by G. KROTKOV, F.R.S.C.

IN recent years emphasis has been placed on the importance of muskeg as a major land type in Canada. Aware of this, the writer and his associates have been concerned with a fundamental method of reference for muskeg. Adequate description of the medium proved difficult because definition must not only relate to material, but also to conditions or states to which the material and the environment contribute separately and in combination. The significance of this complexity has been indicated elsewhere when, for engineers, consideration was given to the principles on which a system for muskeg classification should be built (Radforth, 1952). There, the author proposed and utilized vegetal coverage classes and combinations of these to assist in demonstrating variation in the living component of the organic terrain.

Here, emphasis will be placed on the recognition of botanical relationships in connection with the defining of cover type and subsurface features within the organic terrain. If such relationships among plant components, living and fossilized, are manifest, two major needs will be satisfied. Relationship between characteristics of subsurface and surface organic terrain will be easier to predict and assess, with assurance that generalizations in the field will be more reliable. Also, it will be possible to assess, evaluate, and map conditions associated with terrain change over large areas, particularly for purposes of airphoto interpretation.

FORM DIFFERENTIATION IN VEGETAL COVERAGE

The northern organic terrain providing the greatest number of problems for the field worker falls within the taiga of the Churchill area of Hudson Bay. Between The Pas and the Churchill area in Manitoba for a distance of over five hundred miles, organic terrain is by far the predominating type. South from Churchill on Hudson

Bay, it extends for distances exceeding twenty miles with no interruption and even where finally broken regains characteristic continuity beyond the intrusion.

In these regions, an observer has little difficulty in recognizing a marked peculiarity in vegetal coverage, even though he lacks experience with northern muskeg. There are frequently abrupt character changes in the vegetation. This has the effect of marking out or delimiting discreet areas of vegetation with contrasting character. The observer finds it convenient to make use of these areas as descriptive references. However, when asked to explain the qualities by which detection of difference has been claimed, his reference terms are likely to be ill defined, often intangible, and inconsistent. This is particularly the case when the observer is not trained botanically

Preliminary analysis of the basis leading to detection suggests vegetal form, not species distribution, to be the important agent Sometimes, topographic and other physiographic factors are influential but usually in a secondary sense

Following extensive ground and air survey over wide areas of northern muskeg, the writer has found it convenient to establish a classification system referring to relatively pure categories of vegetation (Radforth, 1952). Nine fundamental classes or coverage types are designated Each is characterized on the basis of variation of three morphological factors—presence or absence of woodiness, range in stature and, where necessary, texture of the foliose material.

Combinations of these class types furnish the descriptive coverage formula for any given area of muskeg Class components, each represented by a letter, are arranged in the formula in order of prominence. If present to a degree less than 25 per cent by inspection of the total coverage, the component is arbitrarily not significant.

It will be appreciated that class combinations are more significant as designators of coverage character than are the pure classes, the elementary constituents. For consideration in this account, coverage combinations have been mapped (Fig. 1). The combination formulae apply to areas sufficiently large to allow for comparisons with terrain character and change in terrain conditions. They are proving useful for geographical demarcation in the field, on maps, or for airphoto interpretation. Also, they are suggestive of natural relationship in the synoecological sense.

Of the nine coverage classes necessary to designate construction of the coverage, four (A, B, D, and E) contain essentially woody plants., The remaining classes (C, F, G, H, I) are non-woody. In the former group, all but the first-mentioned class contain plants less than fifteen feet high. In the latter group no plants exceed a height of five feet and this order of height is rare. Immediately south of the Churchill area, coverage formulae containing Class A (strictly arboreous) or Class B (dwarf tree or tall shrub) may be common (Fig. 2). In parts of the coastal area of Hudson Bay and generally to the north of the arboreous coverage, Classes F, E, H, and I are comparatively prominent (Fig. 1). With the exception of E (shrubs under two feet) all members

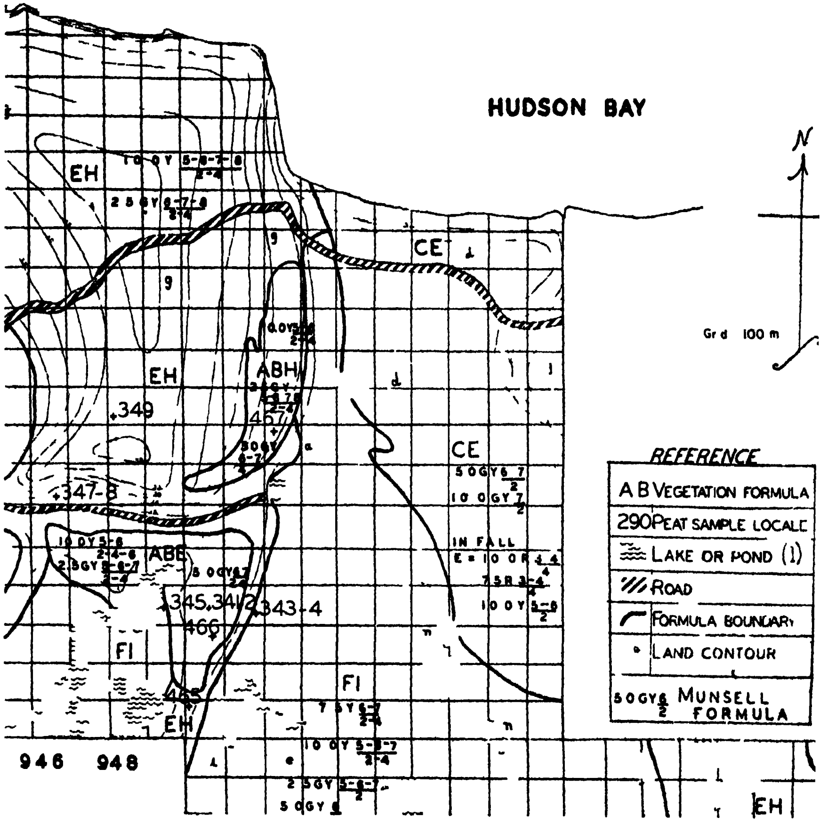


FIGURE 1. Muskeg coverage description map of a coastal area east of Churchill. The spatial relationship between three primary coverage types (E.H., F I, and C.E.) and two secondary ones (A.B.H, A B E.) is expressed.

Topographic symbols (*a*, *d*, *e*, *g*, *i*, *n*) relate to the descriptions established in an earlier work (Radforth, 1952). Among them *d* (rock gravel plain), *g* (exposed boulder), *n* (pond or lake margin sloped) are characteristic of the coverage types with which they occur.

Colour ranges expressed by the Munsell formulae also assist in describing and assessing the coverage character.

of these classes are non-woody: F, eriophoraceous in growth habit; H, lichenaceous, mostly in mats, and I, moss-like, frequently in mats or in hummocks. Beyond the arboreous zone to the south, using the Hudson Bay Railway as a reference axis, treeless barrens are very extensive. Here, combinations of F, E, H, and I cover areas exceeding one hundred square miles, with only negligible interruption by woody plants over five feet in height The common plants making up these cover zones are listed in Table I

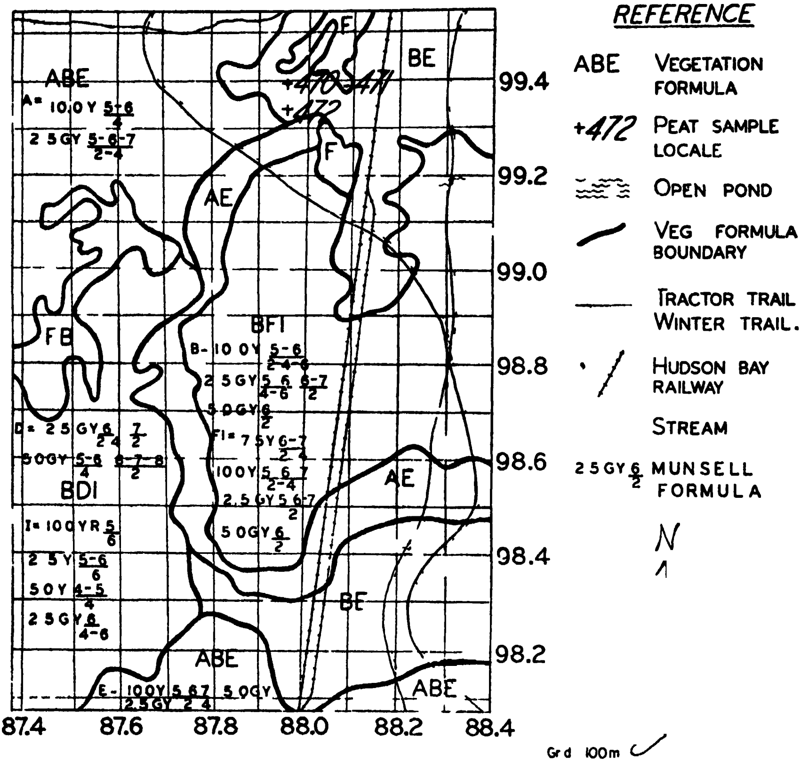


FIGURE 2. Coverage description map for typical muskeg immediately south of Churchill, Manitoba, where dwarf trees and tall shrubs are common (cf Fig 1)

The splendid work of Lewis and Dowding (1926) on floristics of more southern confined muskegs, leads one to the conclusion that, for terrain studies, the concept implied in the expression "ecotone" is highly significant. Sometimes borderlines between associations are sharp, sometimes they are ill defined. This also applies to the more northern muskegs. However, ill-defined ecotones are very common,

TABLE I

Species Predominant in Vegetal Coverage Classes

(Cf. descriptions pages 54–6)

	Class								
	A	B	C	D	E	F	G	H	I
Larix laricina (DuRoi) Koch.	x	xx		x					
Picea glauca (Moench.) Voss	xx	x		x					
Picea mariana (Mill.) B.S.P.	x	xx		x					
Betula glandulosa Michx.		x		xx	x				
Salix spp.	x	xx		x					
Andromeda polifolia L.					xx				
Arctostaphylos rubra (Fern.) Rhed. and Wils.					xx				
Arctostaphylos uva-ursi (L.) Spreng.					xx				
Dryas integrifolia M. Vahl.					xx				
Empetrum nigrum L.					xx				
Kalmia polifolia Wang.				x	xx				
Ledum decumbens (Ait.) Lodd.					xx				
Ledum groenlandicum Oeder				x	xx				
Rhododendron lapponicum (L.) Wahlenb.					xx				
Rubus arcticus L.					xx				
Rubus chamaemorus L.					xx				
Vaccinium uliginosum L.					xx				
Vaccinium Vitis-Idaea L.					xx				
Calamagrostis canadensis (Michx.) Beauv			x						
Carex spp.						xx	x		
Eriophorum angustifolium Roth.						xx			
Eriophorum scheuchzeri Hoppe S. lat.						xx			
Juncus albescens (Lge.) Fern.						xx	x		
Epilobium spp.									
E.g. *E. angustifolium* L.							xx		
Pedicularis spp.									
E.g. *P. labradorica* L.							xx		
Petasites palmata (Ait.) Gray							xx		
Petasites sagittata Pursh.							xx		
Senecio spp.							xx		
E.g. *S. palustris* (L.) Hook.									
Hypnaceae spp.									xx
Sphagnaceae spp.									xx
Cladonia spp.									
E.g. *C. coccifera* (L.) Willd.								xx	
C. fimbriata (L.) Fr.								xx	
C. gracilis (L.) Willd.								xx	
C. rangiferina (L.) Web.								xx	

almost characteristic in the north. Often the gradient between associations is so gradual and extensive that it is hard to recognize a mictium. The dynamic nature of coverage development in the north is made increasingly difficult to understand from the floristic aspect because many species are often found which are common to different ecological circumstances. For these reasons the species represented in Table I are difficult to relate by the usual phytosociological methods unless the concept of "life form" is applied.

The evidence presented here seems to indicate that "communities" as interpreted on a morphological basis are clearly delimited. The relationships they seem to reflect (Figs. 1–3) are natural ones, presumably largely controlled by microclimatological, edaphic, and physiographic conditions.

That species can range into different morphological class zones is not unnatural. Seral development may be proceeding at different rates according to the degree of influence of ecological control. The latter may also be responsible for the localized difference in plant stature applicable to many species. A good example of this is *Ledum groenlandicum* Ceder which appears to have several stature ranges recognizable *en masse* by inspection in the field.

Presence of "form communities" discourages any tendency to regard muskeg coverage as advancing towards a stage when it might be referred to as a "continuum." Subseres, on a form basis, seem to maintain their identity. For those interested in the applied aspects of organic terrain interpretation, this is important. Apparently, discreet zonation of vegetative form may always be expected as a convenience in demonstrating organization and change in the muskeg surface features at all stages of development.

Though it is not within the scope of this account to deal with the use of colour as an interpretive agent in organic terrain studies, secondary reference should be made to its importance. Colour as a variant in terrain coverage character is frequently helpful in identification of class components and delimitation. It is also useful in the comparison of field characters with airphoto records, whether the latter are in colour or not.

The writer finds the best system of colour reference for field use to be the Munsell System (*Munsell Book of Color*) which expresses colour in terms of hue ("relation to red, yellow, green, blue and purple"), value ("lightness") and chroma ("departure from neutral"). Fractions symbolizing values for these components have been used in estimating colour range for different cover assemblages (Figs. 1–3). Seasonal advance must be kept in mind when applying them. However, for a given time, some shift in colour range is recognizable from one cover designation to another. It is these slight characteristic differences which provide useful collateral in large-scale field survey from the air.

PLANT REMAINS AS AIDS IN ORGANIC TERRAIN INTERPRETATION

Fossil plants rank high in significance in that they form the major portion of the mass of material in organic terrain. However, their utilization as reference material is limited, unless organized relationship among them is revealed. Technical field investigators and soil taxonomists tend to describe peaty organic deposits as collections of mosses, leaves, twigs, and roots of higher plants which suggest an unorganized heterogeneous mixture of constituents. Published contributions offsetting this attitude are few. Dachnowski-Stokes (1933) has presented the most encouraging account in this regard and his work demonstrates how peaty deposits may differ on the basis of the predominant constituent species. Others have revealed successional organization in peats based on microfossil determinations. However, the former kind of contribution, scarce at the outset, emphasizes constituent species, not constituent plant form units, and the latter utilizes materials which are largely extrinsic. One contributes to peat genesis on a floristic and ecological basis, the other to interglacial and postglacial history of forests and the distribution of tree species.

Neither type of study, though invaluable in its own sphere, lends itself adequately or directly to investigation of the physical attributes and organization on a form range (gross morphological) basis. Form, size, and spatial relationship of macroscopic constituent particles are as important for the applied studies on peats as for an adequate understanding of mineral soils. Also, microfossils to be useful must reveal organization relative to peaty accumulation. To accomplish this, fossil pollen and spore derivatives of intrinsic source must be emphasized in analyses.

Finally, an approach is required which will be appropriate to an adequate definition of muskeg. This the writer found necessary to define elsewhere as "the term designating organic terrain, the physical condition of which is governed by the structure of the peat it contains, and its related mineral sub-layer, considered in relation to topographic features and the surface vegetation with which the peat co-exists" (Radforth, 1952).

The use of macrofossils for interpretation depends upon the purpose to be served. Presence or absence of large members throughout depth in the organic deposit is important to engineers. Bearing potential and other characteristics will vary with this attribute. Whatever the special need, results depend upon the possibility of classifying the macrofossils as to their size, form, arrangement, and predominance. That variation in size and form does exist will be granted at the outset. How to treat this for classification purposes is not yet fully understood. Variation in arrangement and predominance, if present, may not be revealed on an organized and consistent pattern or series of patterns.

Fortunately, this problem, which is the chief concern of this paper in relation to macrofossils, is not unsurmountable. Its solution lies in the possibility that the record of seral or subseral advance for muskeg is preserved Since most organic terrain is poorly drained and aerated even in its early history, it is a natural medium for fossilization Even should seasonal drying, microclimatic change, or edaphic conditions interfere, some record persists Also, even though certain plants or plant parts disintegrate before or during fossilization leaving no trace of their former presence, field survey indicates that a partial record still remains

To seek evidence for organized pattern in macrofossil constitution by random selection of gross peat samples is undesirable Yet this was the method used in all preliminary surveys The most that the results indicated was confirmation that macroscopically the peats differed widely Some samples consisted of large interlocking woody conglomerations Others had a fine-textured matrix that seemed typical for the designation "black muck" used on occasion to define muskeg. The gradient between these two extremes was marked by numerous examples distinguishable on the basis of particle size, form, arrangement, and predominance

When community development in surface coverage became discernible, search for variation of macrofossil constitution was made accordingly. Significant change in coverage formula was accompanied by evidence of change in macrofossil composition. Though random sampling of organic material was made, it was applied more vigorously wherever surface vegetation pattern changed. Thus, even before discreet surface communities were discerned, indirectly their locations were objectively marked by change in density of subsurface sampling.

As yet quantitative evidence demonstrating macrofossil sample variation with coverage formula is not available. However, descriptive data of a qualitative nature are at hand for several hundred samples. In Fig. 3, coverage formula HE is represented in terms of macrofossil constitution by a predominance of fine-textured, wood-fibrous matrix with associated non-fibrous amorphous matrix. This constitution is interrupted at intervals with discontinuous masses of coarser, mechanically resistant, woody lengths.

For coverage formula AE, the entire mass of peat macroscopically resembles the discontinuous member of HE, except for a foundation of coarse fibrous members varying in size from one-half to one inch in cross-section.

For coverage formula BE, the composition is similar to that for the foundation of AE, with the addition of occasional coarser components as in HE.

Examination of macroscopic content at locations 478 and 479 (Fig. 3), which lie in FI, shows a non-woody fine-textured fibre predominating. This kind of construction is often interrupted by amorphous organic muck and the combined type continues as a background matrix throughout FI, the common coverage for the terrain represented in Fig. 3. Open water occurs only in FI.

Though these descriptions are qualitative in nature they seem to satisfactorily establish the principle that coverage type can be correlated with subsurface macrofossil constitution.

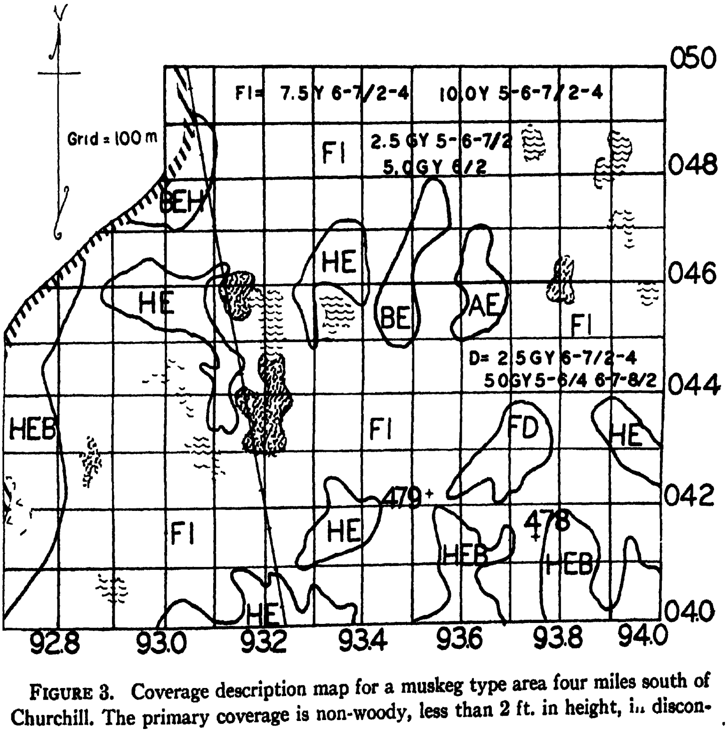


FIGURE 3. Coverage description map for a muskeg type area four miles south of Churchill. The primary coverage is non-woody, less than 2 ft. in height, in discontinuous mats in a mossy matrix.

The level of consistency of the interrelationships expressed is encouraging. This is fortunate, and useful in predicting local terrain conditions. However, there are difficulties, particularly with peat depths exceeding five or six feet. In deep peats, fine-textured, non-fibrous constitution is prevalent in the basal portion which might represent most of the depth of the peat. Also, thickness of fibrous mats and the construction of their components may differ. Spatial relationships between discontinuous secondary units in given types may vary. Similar significant differences occur in given field samples of mineral soils which otherwise compare more or less favourably on a broad basis.

Further evidence of subsurface organization to facilitate prediction of terrain conditions and change rests on the results of microfossil analyses. The writer demonstrated elsewhere (Radforth, 1952) that fossil pollens and spores served as useful indices for reflecting developmental organization in the peat. Seven histogram sets were used. The variants (fossil pollens and spores) were termed index units (Figs. 4, 5). Their frequency was recorded for every two inches of depth as proportions of total counts Frequencies based on counts higher than 200 did not vary appreciably.

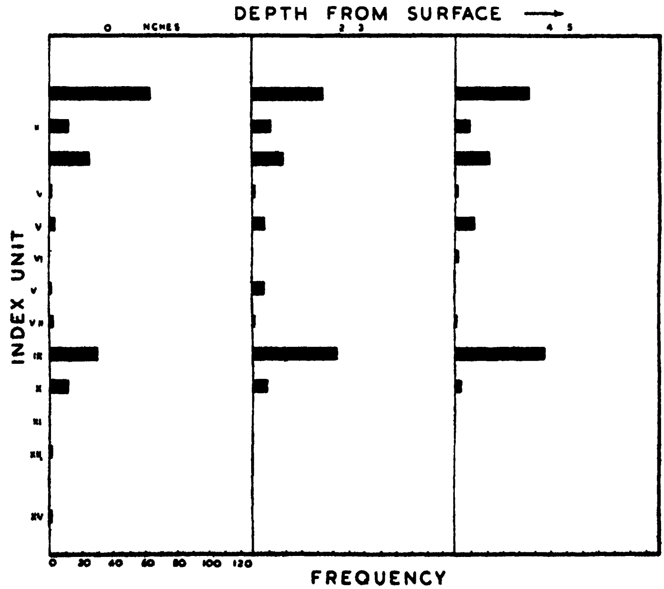


FIGURE 4. Histogram patterns expressing frequency relationships for peats high in Cyperaceous pollens (Index unit IX). The patterns do not change appreciably with depth.

A comparison of histogram results suggests several conclusions:

1. Stable peat constitution throughout depth may occur (Fig. 4).
2. Peats stable in constitution throughout depth may differ in constitution with each other.
3. Some peats, apparently not stable, may reflect a constitutional trend (Fig. 5).
4. Constitutional trends may differ from one example to another.

Intelligent appraisal of these derived observations depends upon a knowledge of the botanical equivalents of the index units, which was not to be found in previous accounts. The botanical designation for each index unit is given in Table II. The somewhat artificial order of listing has been deliberate. It facilitates quick reference in comparing histogram sets.

TABLE II
BOTANICAL DESIGNATION OF INDEX UNITS

Index unit	*Microfossil (pollen or spore)*
I	*Pinus* spp.
II	*Picea glauca*
III	*Picea mariana*
IV	*Alnus*
V	*Betula*
VI	Salicaceae
VII	Ericaceae
VIII	Herbaceous (coverage class G)
IX	Cyperaceae
X	Sphagnaceae
XI	Polytrichaceae
XII	Polypodiaceae
XIII	Equisetaceae
XIV	Lycopodiaceae
XV	Gramineae

Fig. 4, showing a set of histogram patterns which are primarily similar, also shows a high predominance of index unit IX (Cyperaceae) throughout its depth. Where Cyperaceae is relatively high, it is generally the case that there is little evidence for the establishment of a constitutional trend in the peat. When units IV (*Alnus*), V (*Betula*), VI (Salicaceae), VII (Ericaceae), and X (Sphagnaceae) all increase in relation to IX (Cyperaceae) there is also a general similarity of histogram pattern with depth and little suggestion of constitutional change, especially if Sphagnaceae and Ericaceae are unusually high.

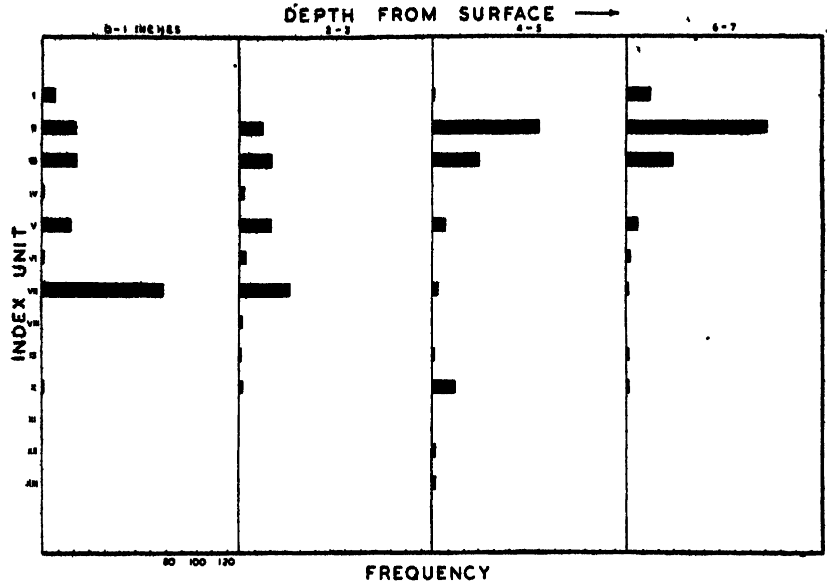


FIGURE 5. Histogram patterns in which relation predominance of index-unit VII (Ericaceae) is gradually achieved with decreasing depth of peat. This type of series occurs with E.H. coverage (Fig. 1) and the peat is relatively well drained.

There are of course exceptions. In the case of Cyperaceous dominance, constitutional trends will be evident in the depth scale if topography is irregular even though the amplitude of contour change is only two to three feet. Such changes sometimes occur at intervals of from twenty to a hundred feet.

A similar situation may arise as an exception when Sphagnaceae and Ericaceae are prominent in the sequence of histograms. However, in this case the amplitude of contour change is greater, from two to five feet, often occurring at intervals from ten to fifty feet apart.

Where, in a given set of histograms, there is dissimilarity in the sequence, the constitutional trend revealed in the peat is reflected in the histogram set as a whole (Fig. 5). Here, Ericaceae has gradually moved into relative prominence at the expense of *Picea glauca*.

In Fig. 6, V (*Betula*) then VI (Salicaceae) increase in relative predominance and IX (Cyperaceae) remains steady. Unit VII (Ericaceae) is not prominent at any depth.

The sets of histograms shown in the text figures represent shallow peats. Sometimes top, uncompressed organic matter is several inches to a foot in depth over the more consolidated organic matter below.

Hence the muskeg as it appears in the field ranges from one to three feet in depth. In muskegs varying from three to six feet in depth, microfossil constitutional trends of a secondary nature may be in evidence. However, primary trends in deep peats showing Cyperaceous prominence at any depth maintain this characteristic at all depths (Fig. 4). When Sphagnaceae, Ericaceae, and Betulaceae show relative predominance over Cyperaceae in deep peats at a given depth, this characteristic is generally maintained at any depth. At the moment of writing, the principles outlined here for shallow peats apparently apply for deep peats with the qualification that, for the latter, secondary constitutional trends may become apparent. These represent a measure of botanical instability in muskeg community development. However, their effect is negligible for purposes of assessing organic terrain variation because they do not appreciably change primary microfossil constitutional stability or trend.

Microfossil studies, in signifying the kind of stability or the kind of trend prevalent in the peaty matrix, provide the most accurate and detailed accounts for organic terrain classification and interpretation. Without this detail, it would be unsafe to predict structural change

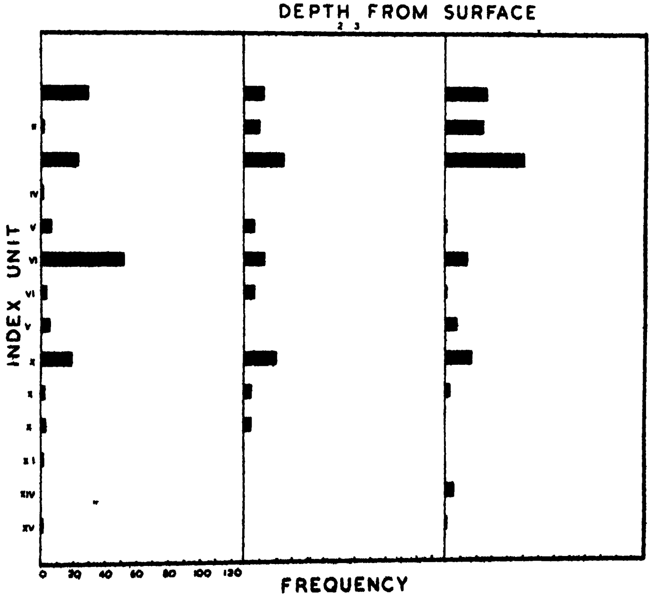


FIGURE 6. Histogram patterns showing change in frequency relationship of microfossil index units with depth This analysis compares favourably with coverage formulae prominent in dense woody constituents, e.g., B.D.I., Fig. 2.

in the terrain. They are also essential to the establishment of a reliable basis of correlation between surface features and macroscopic subsurface constitution.

CORRELATION OF SURFACE AND SUBSURFACE VEGETAL DATA FOR SPECIAL CASES

Though microfossil investigations are fundamental to organic terrain interpretation and to disclosure of organization in peaty material, their contribution is enhanced when considered along with other data. The importance of surface studies and macrofossil survey has already been mentioned. With these it is instructive to correlate any unusual topographic, edaphic, and climatological influence. Also, to assist in aerial interpretation programmes which may follow ground surveys, a knowledge of colour relations in the coverage is essential for best results.

Often a topographic peculiarity in the terrain suggests a problem of muskeg interpretation involving the general appraisal suggested. An example is a type of polygon formation arising frequently in muskeg where permafrost is prevalent. The polygons in Fig. 7 were photographed at an altitude of about 1000 feet when *en route* to Padlei about two hundred miles northwest of Churchill, Manitoba. The detailed study of this type of phenomenon was made about sixty miles to the south of Churchill where the features were typical and the data relatively easy to obtain.

The coverage is also typical for one class of polygon (Figs. 8, 9, 10) Each polygon is ditch-like and along with neighbouring polygons forms a network of depressions, often with abrupt walls except where wind action has uncovered the dead organic remains on the shoulders (Fig. 8). Segments of two depressions are shown in Figs. 8 and 9 with metre quadrat stakes in place. The polygon encloses a peaty plateau with hummocks frequenting the marginal zone (Fig. 10).

The coverage in the depressions contrasts markedly with that on the plateaus. Usually for the former the coverage class is FI and less frequently FD (Figs. 8, 9). For the plateaus the coverage is HE (Fig. 10). The macrofossil constitution throughout depth compares favourably with normal HE coverage (see page 60), except for the upper third (about one foot) of the peat in the depressions where it largely resembles that for FI elsewhere (see page 61). Occasionally where D class becomes influential, the coarse wood-fibrous element invades the peaty matrix.

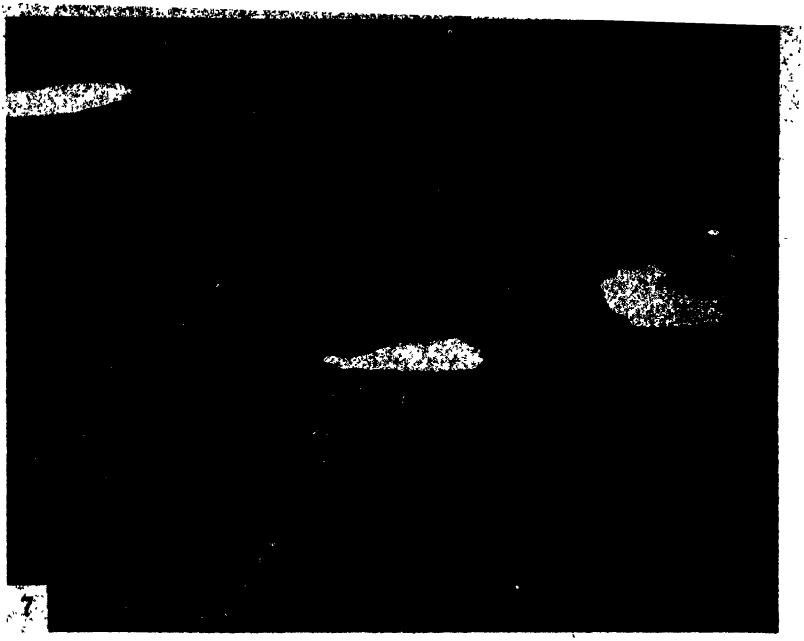
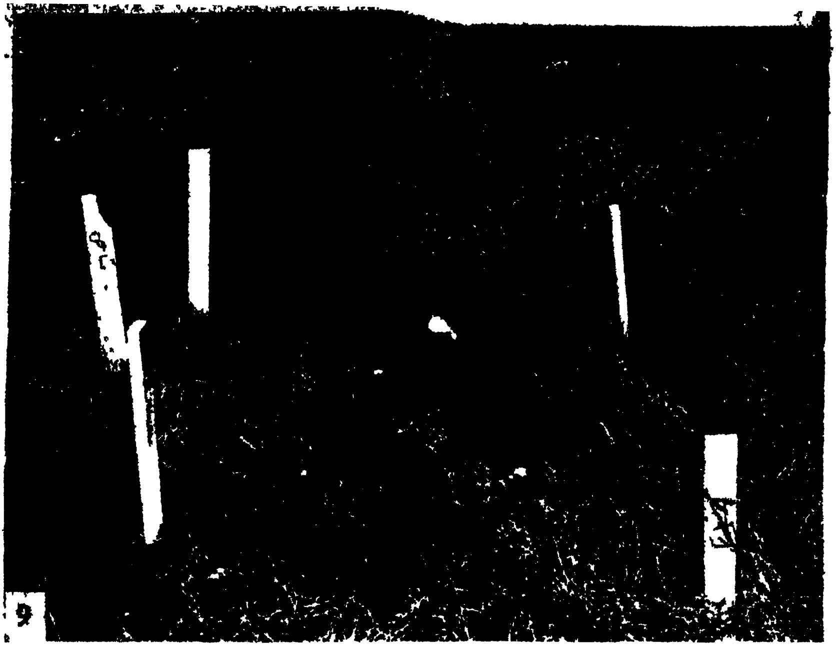


Figure 7. Aerial photograph taken at an altitude of about 1,000 feet *en route* to Padlei from Churchill, showing polygon formation in the foreground.

Figure 8. A polygon marginal depression showing some F.D. coverage and exposed eroded shoulders of dead organic material.



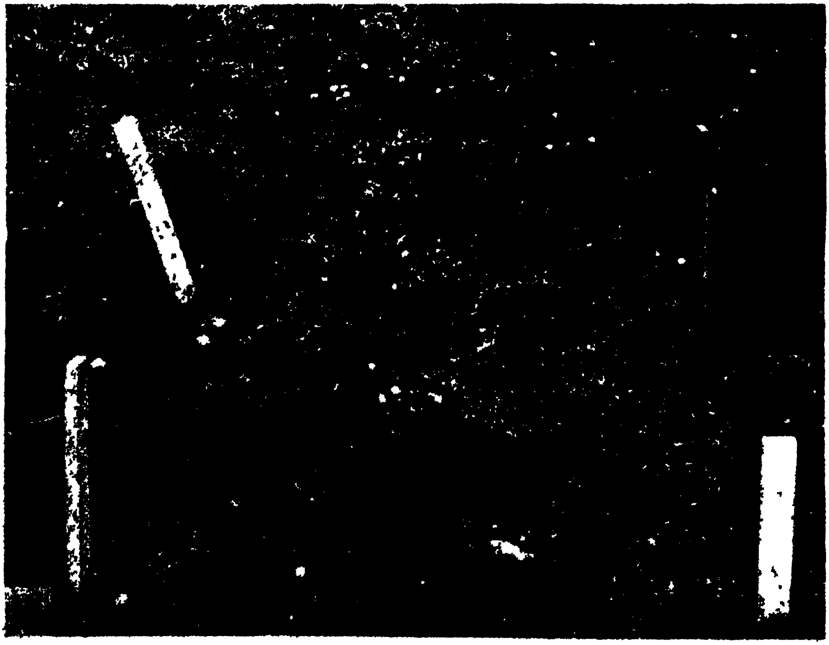


Figure 9. A polygon marginal depression showing F I. coverage Drainage here is poor as compared with that for F.D coverage.

Figure 10. A type polygon enclosure demonstrating frequent hummocking which occurs with H.E. coverage in the polygon plateaus thirty miles south of Churchill.

Microfossil analysis in the plateaux shows a uniform constitution comparing favourably with that described on page 60 for HE. In the depressions a trend is exhibited in the upper portion in the direction of the constitution described for FI.

The second category of polygons is recognized at once by F and FI class coverage for the plateaux with macrofossil and microfossil constitutions typical for FI coverage. In the depressions the coverage class is DF in the Churchill area, changing to F or I towards Padlei (Fig. 7). For the DF coverage, in the depressions the macro-microfossil constitution exhibits trend; for the F or I class, no trend.

This kind of comparison facilitates conclusions concerning the constructional properties of polygon terrain and its associated physiographical character. The terrain shown in Fig. 10 with HE coverage has with its woody-fibrous constitution relatively good resistance to compressional force and is reasonably well drained. It has a high degree of irregularity topographically, as compared with the type with FI coverage. For the former type the terrain has resilience in contrast with the latter which tends to lose coherence and to dissociate structurally when disturbed

Comparisons on the basis of coverage alone, though very helpful, are not adequate for predicting polygon terrain conditions. Neither is the combination of coverage and macrofossil constitution. Where class D is promiment in the depressions there is a reasonable mechanical stability to the terrain for the first type of polygon, but not for the second. Drainage is good in the first and poor in the latter. Differentiation between these two on the basis of plant material is only reliable through microfossil analysis. For the relatively stable, better-drained example with DF coverage, the macrofossil constitution will be similar to that for the unstable example. The microfossil histogram pattern would show a relatively high density of Ericaceae-Betulaceae for the approximate lower two thirds of the peat in the case of the former and a relatively high Cyperaceous density for the latter.

SUMMARY AND CONCLUSIONS

The occurrence of zonation in coverage vegetation provides a valuable aid in interpreting and assessing organic terrain character. In addition, the utilization of zone limits for mapping facilitates an appreciation of terrain change and frequency of change

It is also significant that the zonation is not based on arbitrary factors and classification technique. The recognition and use of "form communities" rather than floristic communities does not affect this claim. The grouping of form types as they occur naturally provides a logical lead for subsurface exploration. It also suggests the means by which frequency and distribution of terrain differences may be assessed on an organized basis, from the ground or the air, particularly when vegetation colour range is an accessory interpretive agent.

In only two combinations of coverage classes (HE and FI) does macrofossil constitution seem to be predictable and consistent throughout the depth of the peat. Also, macrofossil constitution for extensive areas is difficult to characterize from small gross samples. This makes terrain interpretation difficult and uncertain unless microfossil constitution is considered.

Microfossils provide the ultimate source of reference and the most reliable source of plant materials in the investigation of northern organic terrain. They are indicators of organization for the vertical and horizontal axes in the organic matter. Through them, detection of constitutional change in peats can be accomplished and assessed. Indirectly they are aids in the study of drainage and other physiographic features.

Finally, and most significantly, microfossils are essential to an understanding of botanical and structural development anywhere in the muskeg. In this capacity, they support the use of vegetal coverage in the classification and prediction studies of terrain interpretation. This is subject to the condition that laboratory analyses of microfossil sequence corresponding to given coverage combinations are consulted fully.

ACKNOWLEDGMENTS

The writer is indebted to Miss Jean Evel for her assistance in the preparation of this manuscript and to Edward J. Johnson who as a research technician has provided many of the technical data. Grateful acknowledgment is also extended to many students who have contributed to muskeg studies and to several colleagues, particularly Robert F. Legget, Director, Division of Building Research, National Research Council, to whom all credit must go for initiating inquiry into organic terrain constitution. The National Research Council through the Associate Committee on Soil and Snow Mechanics has provided financial aid along with the Defence Research Board,

through the Arctic Research Section The latter has shown keen interest in delimitation of muskeg surface character in aerial interpretation studies presently being conducted by the writer.

REFERENCES

Auer, Vaino (1933) Peat bogs of southeastern Canada Handbuch der Moorkunde **7** 141–223

Dachnowski Stokes, A P (1933) Peat deposits in U S A Handbuch der Moorkunde **7** 1–140

Lewis F J and Dowding F S (1926) The vegetation and retrogressive changes of peat areas (' muskegs) in central Alberta J Ecol, **14** (2) 317–41

Munsell Book of Color Pocket ed, Baltimore Munsell Color Co Inc 1929–42

Radforth N W (1952) Suggested classification of muskeg for the engineer Engineering J, **35** (11) 1–12

Date of issue	Date of issue	Date of issue
10.5 60		
3-6-60		
11/11/66		
18.4.67		

GIPN—S4—34 I. A R I. 56.—22-4-57—4,000.